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DOLICHODORUS PROFUNDUS N. SP. (NEMATODA - TYLENCHIDA)

PAR

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Au cours de l'année 1958, près de 150 échantillons de sol provenant des cocoteraies du Togo furent analysés en vue de reconnaître un rôle éventuel des nématodes phytoparasites dans la „maladie de Kaïncopé" qui ravage les plantations de cocotiers de cette partie de la côte du Benin et dont la cause demeure inconnue.

Les prélèvements de sol étaient habituellement effectués à 20-25 cm de la surface. Cependant, pour connaître le répartition verticale des nématodes phytoparasites, deux séries de prises échelonnées en profondeur de 40 en 40 cm, de la surface du sol à la nappe phréatique, eurent lieu. Les nématodes phytoparasites communs dans ce type de sol (*Xiphinema, Scutellonema, Hemicycliophora,* etc.) n'étaient abondants que dans la zone de 0 à —40 cm, très rares dans la zone —40 à —80 cm et n'étaient pas représentés dans les zones plus profondes; ceci est logique car la majorité des racines de cocotier, seul végétal présent à l'endroit du prélèvement, forme un lacis dense entre les niveaux —5 et —50 cm. Par contre dans deux de ces trois séries de prélèvements furent rencontrés quelques individus d'un *Dolichodorus*: à —120 cm (nappe phréatique à —160 cm) et —100 cm (nappe phréatique à —120 cm); ces individus, appartenant à une nouvelle espèce décrite ici sous le nom de *Dolichodorus profundus* n. sp., nom tiré précisément de son biotope, n'ont été rencontrés ni dans les couches plus superficielles du sol ni au niveau même de la nappe phréatique. Ils semblent donc vivre dans les sables humides, profonds, vraisemblablement aux dépens du cocotier dont certains faisceaux de racines plongent jusqu'à la nappe phréatique.

L'existence de cette espèce dans les couches profondes du sol, et uniquement dans celles-ci, conduit à penser qu'une image complète du peuplement nématologique parasite d'une culture arbustive ne peut être donnée par les seuls prélèvements effectués dans les couches superficielles du sol, même si celles-ci contiennent la grande majorité des racines.

D. profundus n. sp. semble toutefois rare car malgré le passage de plusieurs kilogs de sol aux élutriateurs de Seinhorst, seuls six femelles, trois mâles en un certain nombre de juvéniles purent être récoltés.

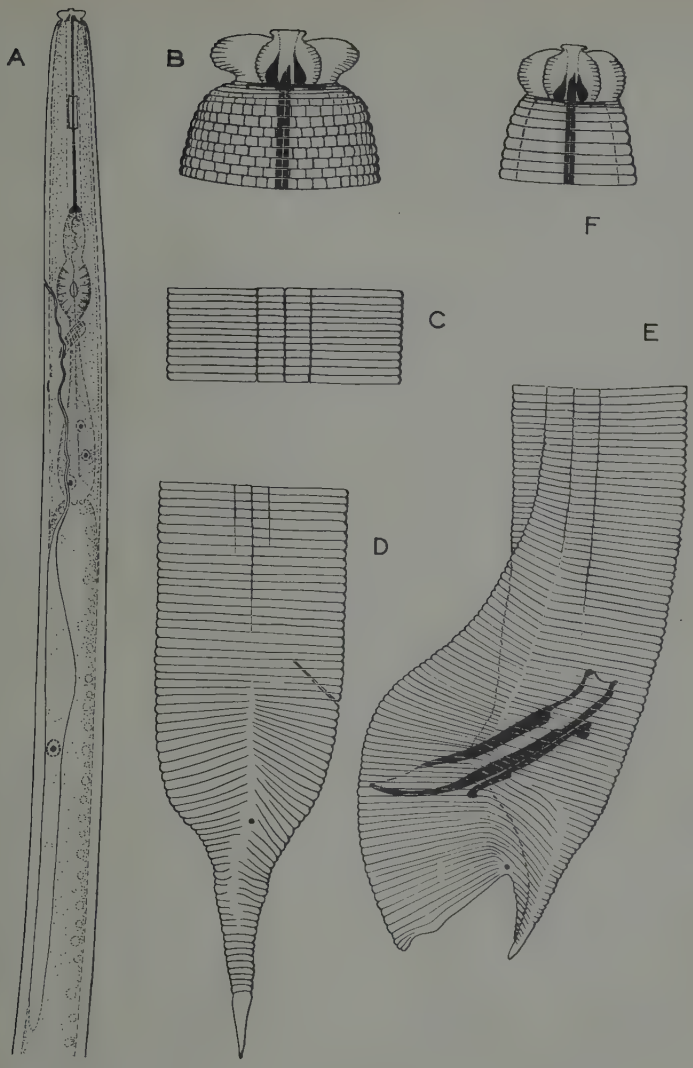
En dehors de la morphologie externe bien particulière de la région céphalique qui sera détaillée plus loin, cette espèce est remarquable par la structure de son

appareil excréteur. Au pore excréteur, très antérieur, fait suite un canal à paroi sclérotisée, de 1,5 μ de diamètre, très sinueux et ne semblant pas noyé dans une des cordes latérales comme il est admis classiquement (CHITWOOD & CHITWOOD 1950) pour les Tylenchida mais plutôt libre et au contact direct de l'oesophage. Cette structure ne semble d'ailleurs pas exceptionelle et M. W. Allen nous signale (*in litt.*) l'avoir observée chez une demi-douzaine de genres appartenant aux Tylenchida. Sur la plupart des individus on peut suivre le canal excréteur jusqu'à environ un diamètre correspondant en arrière du bulbe oesophagien médian; à ce niveau le canal, en position ventro-latérale gauche, fait place à une structure particulière: il s'agit d'une sorte de sac très allongé (jusqu'à 300 μ), à paroi non sclérotisée, mince et difficilement perceptible, s'élargissant progressivement jusqu'à près 20 μ à son tiers antérieur puis diminuant de diamètre et se poursuivant rectiligne. Le contenu en est hyalin, réfringent, homogène, avec de grosses vacuoles peu nombreuses et l'on y distingue un seul gros noyau, situé à moitié environ; la limite postérieure de cette structure est peu nette et il est possible qu'elle se poursuive en arrière, avec un diamètre alors très réduit. La présence d'un noyau et de parois non sclérotisées fait penser plus à une glande qu'à un simple élargissement du canal excréteur.

Il est possible, comme le suggère l'anatomie de *Paraphelenchus myceliophthorus* Goodey, 1958, récemment étudiée par GOODEY (1958, 1959), que la glande décrite ne soit pas la prolongation directe du canal excréteur mais que celui-ci se poursuive en arrière indépendemmant d'elle; si une semblable structure de l'appareil excréteur n'a pu être observée, la difficultée des observations au niveau même de la junction de la glande ne permet pas de conclure de façon certaine sur ce point.

Chez *D. heterocephalus* Cobb, 1914 une structure de l'appareil excréteur analogue à celle de *D. profundus* n. sp. a été observée; elle avait d'ailleurs été figurée par Cobb dans l'article original, bien que le texte n'apporte malheureusement aucune précision sur ce point.

Par contre nous n'avons pu observer cette structure ni chez *D. obtusus* Allen, 1957, ni chez *Macrotrophurus arbusticola* Loof, 1958, genre voisin de *Dolichodorus,* mais nous ne pouvons affirmer non plus qu'elle n'y existe pas. Cette structure ne s'observe aisément en effet que chez les individus presque entièrement privés des globules très réfringents qui garnissent habituellement les cellules intestinales, ce qui n'était pas le cas des individus examinés appartenant à ces deux dernières espèces. Il est possible également que la technique de montage rapide employée pour nos lames (SEINHORST 1959) soit plus favorable à l'examen de l'appareil excréteur que la technique lente classique.



Pl. 1. — *Dolichodorus profundus* n. sp. — Femelle. A: portion antérieure du corps montrant la structure de l'oesophage et de l'appareil excréteur — B: tête — C: champ latéral un peu avant la vulve. D: queue — Mâle E: queue.

Doliochodorus heterocephalus Cobb 1914 — Femelle. F: Tête. (gross.: A: 260 — B, C, D, E, F: 1150).

Dolichodorus profundus n. sp.

Dimensions: Pl. 1: A-E

Femelles (6) L = 2,11-2,49 mm; a = 42-53; b = 8,2-10; c = 30-44; V = $^{14\text{-}24}$ 50-54 % $^{15\text{-}19}$.

Mâles (3) L = 2,22-2,37 mm; a = 48-68; b = 8,1-8,5; c = 61-87; T = 56-72 %.

Holotype: ♀ L = 2,49 mm; a = 50; b = 9,2; c = 34; V = ^{14} 52 % ^{15}.

Allotype: ♂ L = 2,37; a = 68; b = 8,5; c = 61; T = 72 %.

Description:

Femelles: Lorsqu'elles sont tuées lentement par la chaleur les femelles adoptent un habitus rectiligne. Corps cylindrique, épais, rapidement aminci vers l'avant et vers l'arrière. Cuticule marquée par des anneaux de 1,4 μ de large en moyenne au milieu du corps; vers l'avant les anneaux sont divisés par des stries longitudinales en petits rectangles très irréguliers, non situés en files longitudinales; cette ornementation particulière s'affaiblit au niveau de la base du stylet et s'évanouit à celui du bulbe oesophagien médian. Le champ latéral est marqué par trois lignes longitudinales équidistantes traversées par les anneaux (champ latéral aréolé); au centre du corps il occupe un peu moins du quart du diamètre correspondant; la naissance antérieure de la ligne médiane du champ latéral est difficile à percevoir car elle se confond avec les séparations longitudinales des rectangles cuticulaires décrits plus haut; elle se poursuit sur tout le corps et ne disparait qu'après l'anus; les lignes latérales apparaissent au niveau du bulbe oesophagien médian et disparaissent à environ une largeur anale en avant de l'anus. Deirides non observées. Phasmides ponctiformes, situées un peu postérieurement au tiers antérieur de la queue. Queue de longueur variable (54 à 72 μ), d'abord régulièrement conique puis se rétrécissant rapidement pour se terminer par une pointe effilée.

Région labiale turbiniforme, aplatie vers l'avant; disque labial bien marqué, occupant un quart de la largeur labiale; apparemment 4 lèvres comportant 10-12 anneaux très fins, difficilement perceptibles; lèvres séparées du corps par une gorge large et profonde; à ce niveau les anneaux du corps sont plus étroits; sclérotisation céphalique massive; plaque basale très nette. Stylet fin, de 105-117 μ de long, le protorhabdion occupant environ les deux tiers de cette longueur; boutons basaux arrondis, dirigés vers l'arrière. Guide du stylet tubulaire, situé à moitié du stylet environ, à anneaux larges, long de 25-28 μ. Ouverture de la glande oesophagienne dorsale à 4 μ de la base du stylet. Procorpus renflé, à lumière sineuse; bulbe médian ovale, musculeux, occupant la moitié du diamètre correspondant; isthme d'abord étroit et passant graduellement à un bulbe basal glandulaire renflé, trilobé (ces lobes ne se terminant pas toujours exactement au même niveau) et contenant trois noyaux inégaux: un gros généralement situé à la moitié et deux noyaux plus petits situés postérieurement. Cardia étroit et bas.

Anneau nerveux situé derrière le bulbe oesophagien médian. Hemizonide large et plat, occupant la largeur de six anneaux environ, à 195-230 μ de l'avant. Pore excréteur à 144-164 μ de l'avant, au niveau de la moitié antérieure du bulbe

médian; aucune ampoule excrétrice observée; canal excréteur fin, de 1,5 μ de diamètre, à paroi très sclérotisée, permettant de suivre facilement son trajet; à un diamètre correspondant environ en arrière du bulbe postérieur oesophagien il fait place à la structure particulière décrite plus haut.

Vulve située à peu près au milieu du corps (49,8-53,5 %), en fente profonde. Deux ovaires droits, avec ovocytes sur une seule file. Spermathèque grossièrement arrondie. Oeufs non observés.

Mâles: Semblables à la femelle. Habitus en crochet, la moitié postérieure du corps étant recourbée en demi-cercle. Bourse trilobée caractéristique du genre, crénelée, enveloppant l'extrémité de la queue; phasmides situées dans les échancrures latérales de la bourse. Spicules épais, très peu arqués, longs de 46-51 μ; gubernaculum long de 25-28 μ, mince, à extrêmité distale légèrement épaissie et extrémité proximale présentant un bouton très net Testicule unique. Spermatozoïdes sphériques, de 2 μ de diamètre environ.

Localité type: au voisinage de racines de *Cocos nucifera* L., à 1,2 m de profondeur; Akodessewa. Togo.

Holotype: Femelle — Lame 23-To-99-Laboratoire de Nématologie I.D.E.R.T. — Abidjan (Côte d'Ivoire).

Allotype: Mâle — Lame 322-To-215-Laboratoire de Nématologie I.D.E.R.T. — Abidjan (Côte d'Ivoire).

Diagnose: *D. profundus* n. sp. se différencie des trois espèces du genre déjà décrites (*D. heterocephalus* Cobb, 1914, *D. obtusus* Allen, 1957, et *D. similis* Golden, 1958) par la forme particulière de ses lèvres et l'ornementation cuticulaire de sa partie antérieure. Chez les trois autres espèces en effet les lèvres, plus ou moins sphériques, sont séparées du corps soit par une incisure moyenne et ornée d'anneaux bien visibles (*D. obtusus*), soit par une incisure plus nettement marquée et ornée d'anneaux fins (*D. heterocephalus* et *D. similis*). Aucune ne possède les lèvres typiques de *D. profundus,* non hémisphérique mais aplaties vers l'avant, en forme de bourrelet, et séparées du corps par une gorge profonde du sixième de la plus grande largeur labiale. L'ornementation cuticulaire en petits rectangles de la partie antérieure du corps est également particulière à *D. profundus*. Ces deux caractères sont les seuls qui séparent cette espèce de *D. heterocephalus*. Elle se différencie de plus de *D. similis* par son stylet plus long (105-117 μ contre 79-87 μ) et son pore excréteur situé plus antérieurement, au niveau du bulbe oesophagien médian et non à celui du bulbe basal, et de *D. obtusus* par la forme de sa queue, pointue et non arrondie, et son champ latéral à trois incisures au lieu de quatre.

Nous remercions très vivement M. H. Hoestra qui nous a fait parvenir les prélèvements de sol, le Dr. G. J. Rau et le Dr. A. M. Golden pour l'envoi d'échantillons fixés de *Dolichodorus heterocephalus,* M. P. A. A. Loof pour le prêt de lames de *Macrotrophurus arbusticola* et enfin le Dr. M. W. Allen qui nous a transmis des lames de *D. obtusus* et fait part de ses observations sur la structure de l'appareil excréteur chez les Tylenchida.

SUMMARY

Dolichodorus profundus n. sp. (Nematoda-Tylenchida)

The author describes and figures *Dolichodorus profundus* n. sp. This new species was found in the sandy soil of a coconut plantation near Akodessewa (Togo) at a depth of 1 to 1.2 m (0.2 to 0.4 m above the soil water table) and never in the more superficial layers of the soil, whereas other plant-parasitic nematodes found in this type of soil are more prevalent in the upper layers. *D. profundus* n. sp. differs from the other species of the genus by not having more or less hemispherical lips but cushion-shaped lips separated from the body by a very broad and deep groove. In addition the cuticle, in the fore part of the body only, is marked by minute rectangular plates instead of plain annules. A particular structure of the excretory system, consisting in a very long uninucleate, left sub-ventral cell joined to the excretory duct, was observed both in *Dolichodorus profundus* n. sp. and *D. heterocephalus* Cobb, 1914.

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EDITORS' NOTE

The Editors have pleasure in announcing that Dr. D. J. Raski, University of California, Department of Nematology, Davis, California, has agreed to join the Editorial Board. As from this issue will contributors from North and South America please send one script directly to Dr. Raski and one to Dr. van der Laan.

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TROIS NOUVELLES ESPÈCES DU GENRE *ROTYLENCHOIDES* WHITEHEAD, 1958 (NEMATODA - TYLENCHIDA)

PAR

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Quand parut l'article de WHITEHEAD (1958) donnant la description du genre *Rotylenchoides*, l'auteur avait en cours l'étude d'une nouvelle espèce appartenant à ce même genre, *R. intermedius* n. sp. Depuis, deux autres espèces ont été rencontrées qui sont décrites ici sous les noms de *R. variocaudatus* n. sp. et *R. affinis* n. sp. L'étude de ces trois nouvelles espèces permet de mieux saisir la conception du genre *Rotylenchoides*.

R. intermedius n. sp. très proche morphologiquement de *R. brevis* Whitehead, 1958 s'en différencie par un caractère important du tractus génital femelle. La gonade postérieure en effet au lieu d'être réduite à un court sac post-utérin peut atteindre une longueur de 55 μ et se composer d'une file de petites cellules apparemment indifférenciées et à noyau indistinct; on y reconnait ni ovaire, ni spermathèque, ni cellules de l'oviducte comme dans la gonade antérieure; il s'agit là d'une gonade régressée, non fonctionnelle, mais qui marque de façon tangible la proximité entre les genres *Rotylenchoides* et *Helicotylenchus*; corrélativement la vulve est située plus antérieurement que chez l'espèce type. Les mâles de cette espèce sont très semblables à ceux de *R. brevis*.

R. variocaudatus n. sp. et *R. affinis* n. sp. ne diffèrent entre eux que par de légers détails; tous deux possèdent une gonade postérieure femelle réduite à un sac post-utérin, comme chez *R. brevis*. Chez ces deux espèces seules des femelles ont été observées; la présence dans les deux cas d'une spermathèque remplie de spermatozoïdes laisse supposer l'existence de mâles mais malgré l'analyse de plusieurs kilogs de sol aucun n'a été rencontré. Chez ces deux espèces le queue n'est pas hémisphérique mais conique et plus ou moins allongée.

Le genre *Rotylenchoides* ne possède en propre aucun caractère particulier; il emprunte les uns et les autres tantôt aux Hoplolaiminae typiques tantôt au genre *Pratylenchus*. Examinons ces différents caractères.

Chez les trois espèces décrites dans le présent article, la partie basale de l'oesophage recouvre l'intestin ventralement ou latéroventralement, les trois lobes glandulaires étant parfois légèrement détachés les uns des autres. Chez *R. brevis*, Whitehead nous a confirmé (*in litt.*) que ce recouvrement était plutôt latéral que dorsal ou ventral. Ce caractère rapproche les *Rotylenchoides* du genre *Praty-*

lenchus où le recouvrement est ventral ou latero-ventral mais jamais dorsal et l'éloigne des Hoplolaiminae typiques où le recouvrement dorsal est de règle. La queue de *R. variocaudatus* avec ses phasmides situées postérieurement à l'anus et sa forme allongée ressemble également quelque peu à une queue de *Pratylenchus*. Enfin la présence d'une seule gonade femelle fonctionnelle est un caractère commun avec *Pratylenchus* alors que les Hoplolaiminae typiques ont toujours deux gonades femelles fonctionnelles.

Quelle est la valeur de ces caractères? Le recouvrement ventral de l'intestin par la partie basale de l'oesophage peut exister chez *Helicotylenchus* où nous l'avons observé chez une espèce indéterminée de ce genre; d'autre part s'il est la règle dans le genre *Pratylenchus,* chez *Radopholus* ce recouvrement peut indifféremment être ventral (*R. gracilis* (De Man, 1880) Hirschmann, 1955; *R. lavabri* Luc, 1957) ou dorsal (*R. similis* (Cobb, 1915) Thorne, 1949; *R. inaequalis* Sauer, 1958). Des rapprochements tirés de l'absence de telle ou telle structure, ici la gonade postérieure femelle, ne peuvent fort bien révéler qu'une convergence; d'autre part la présence chez *Rotylenchoides intermedius* n. sp. d'une gonade postérieure femelle réduite montre la transition vers les espèces didelphiques.

Beaucoup plus importants nous paraissent les caractères tirés de l'examen de la partie antérieure du corps. L'analogie de structure avec les Hoplolaiminae typiques est complète: lèvres arrondies, non séparées du corps, comportant 4 anneaux, sclérotisation céphalique très développée; stylet long, à boutons basaux non arrondis antérieurement et présentant parfois un procès antérieur net, ouverture de la glande oesophagienne dorsale à une certaine distance du stylet; ce dernier caractère, s'il n'est pas constant chez les Hoplolaiminae ne se retrouvant jamais chez les Pratylenchinae. La ressemblance est telle qu'il est presque impossible de reconnaître un mâle de *Rotylenchoides* d'un mâle de *Helicotylenchus,* hormis l'indication donnée par le recouvrement oesophago-intestinal.

C'est pourquoi l'existence de la sous-famille des Rotylenchoidinae qui se justifiait au seul examen de *R. brevis,* et ne se basait en fait que sur l'absence d'ovaire postérieur, semble précaire après l'étude des nouvelles espèces, notamment de *R. intermedius*. Il nous semble que la place du genre *Rotylenchoides* est plutôt dans la sous-famille des Hoplolaiminae où il occuperait à coté du genre *Helicotylenchus* une position homologue à celle du genre *Pratylenchus* à coté de *Pratylenchoides,* ces deux genres dans la sous-famille des Pratylenchinae. Certes il faudrait pour cela modifier la diagnose des Hoplolaiminae mais le Dr S. A. Sher travaillant actuellement à une révision de ce groupe il nous paraît prématuré d'y procéder nous-même [1]).

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[1]) Le nouveau genre *Hoplotylus* récemment décrit (s'Jacob, J. J. (1959) — *Hoplotylus femina* n.g., n.sp. (Pratylenchinae; Tylenchida) associated with ornamental trees — *Nematologica,* **4**, 317-321), fait également transition entre les Hoplolaiminae et les Pratylenchinae typiques. *H. femina,* l'espèce unique du genre, est proche de *Rotylenchoides variocaudatus* n. sp. et *R. affinis* n. sp. par la forme générale du corps et celle de la queue, mais s'en différencie par un stylet moins développé et une portion oesophagienne basale recouvrant dorsalement et sur une grande longueur la partie antérieure de l'intestin. (Note ajoutée sur épreuve).

Cependant il est nécessaire d'amender la diagnose du genre *Rotylenchoides* pour y faire entrer les trois espèces décrites dans cet article.

Genre *Rotylenchoides* Whitehead, 1958

Diagnose amendée — *Hoplolaiminae* (Filipjev, 1934) Thorne, 1949

„Tête avec six lèvres, non séparées du corps; sclérotisation céphalique très développée. Cuticule annelée. Champ latéral marqué par des incisures longitudinales. Stylet long, épais, avec des boutons basaux bien développés, non arrondis. Oesophage avec un bulbe médian ovale et une partie glandulaire postérieure recouvrant la portion antérieure de l'intestin. Phasmides ponctiformes. Femelles avec la seule gonade antérieure développée et fonctionnelle; gonade postérieure non fonctionnelle, réduite à une file de cellules indifférenciées ou à un sac postutérin de longueur variable. Bourse et gubernaculum présent· un telamon peut exister."

(Espèce type: *Rotylenchoides brevis* Whitehead, 1958).

Clé des espèces du genre *Rotylenchoides*

1 — Queue de la femelle hémisphérique — Mâles connus 2
— Queue de la femelle allongée, plus ou moins régulièrement conique — Mâles inconnus . 3
2 — V = environ 90% — Spicules: 21-25μ . . . . . *brevis* Whitehead, 1958
— V = environ 80% — Spicules: 14-15 μ . . . . . *intermedius* n. sp.
3 — Phasmides situées aus $^2/_5$ antérieurs ou à la moitié de la queue — Lignes centrales du champ latéral ne fusionnant pas ou très postérieurement . . . . . *variocaudatus* n. sp.
— Phasmides situées au niveau de l'anus ou très peu postérieurement — Lignes centrales du champ latéral fusionnant en une ligne unique à mi-longueur de la queue . . . . *affinis* n. sp.

Rotylenchoides intermedius n. sp.

Dimensions: 6 ♀♀: L = 0,394-0,523 mm (moy. = 0,454 mm)
a = 22,4-25,5; b = 3,9-4,7; c = 41,4-59,7
V = $^{27,3\text{-}30,9}$ 78,0-83,2 % $^{10,4\text{-}12,1}$
3 ♂♂: L = 0,343-0,387 mm (moy. = 0,372 mm)
a = 25,4-29,7; b = 3,7-4,5; c = 25,7-32,2
T = 30,6-56,3 %

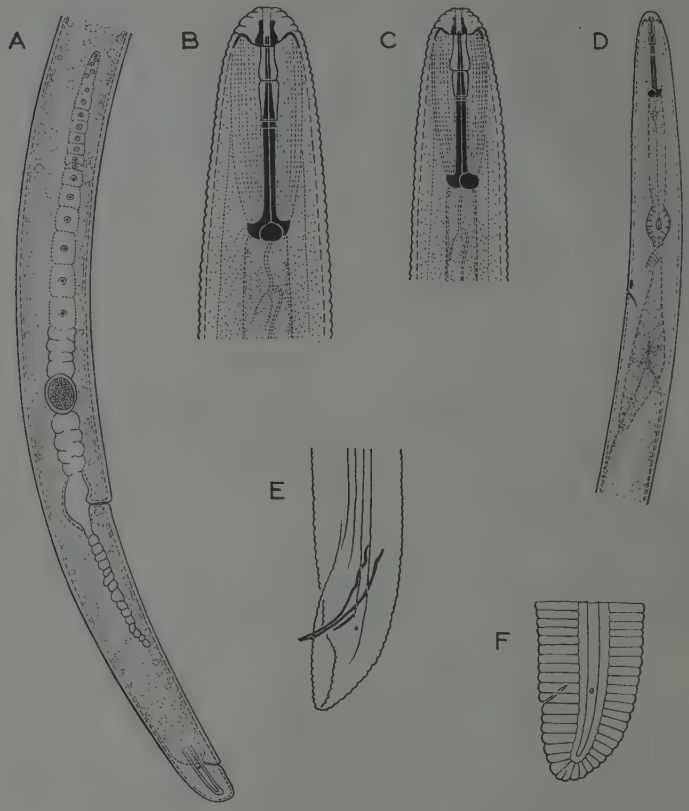
Holotype: ♀: L = 0,436 mm; a = 23,5; b = 4,4; c = 48,6
V = ^{28,6} 78,9 % ^{12,1}

Allotype: ♂: L = 0,387 mm; a = 29,7; b = 4,3; c = 32,2
T = 53,6 %

Description:

Femelles: Habitus presque rectiligne ou légèrement courbé (sur animaux tués lentement à la chaleur). Corps peu effilé vers l'avant; rétrécissement marqué au niveau de la vulve; léger rétrécissement seulement à celui de l'anus.

Lèvres hémisphériques, comportant 4 anneaux, non séparées du corps. Squelette céphalique bien développé; plaque basale s'étendant en arrière sur 3 anneaux du corps. Stylet long de 26-27 μ, le protorhabdion étant légèrement plus court que le telorhabdion; boutons basaux bien développés et détachés du corps du



Pl. 1. — *Rotylenchoides intermedius* n. sp. — Femelle: A: portion postérieure du corps montrant la structure du tractus génital. B: tête. F: queue. — Mâle: C: tête. D: portion antérieure du corps. E: queue. (gross.: A, D: 570 — B, C, E, F: 1150).

stylet avec une partie postérieure arrondie et une partie antérieure concave; procès antérieur à peine esquissé. Muscles protracteurs puissants.

Procorpus cylindrique. Bulbe médian ovale. Portion glandulaire basale trinuclée recouvrant ventralement ou ventro-latéralement la partie antérieure de l'intestin. Orifice de la glande oesophagienne dorsale débouchant à 7-9 μ de la base du stylet. Intestin sans caractère particulier.

Anneau nerveux situé immédiatement sous le bulbe médian. Pore excréteur à 78-98 μ de l'avant. Hemizonide situé immédiatement en avant du pore excréteur et s'étendant sur deux anneaux.

Vulve située postérieurement, à 80 % du corps en moyenne, en fente profonde; tractus génital antérieur atteignant 111-162 μ de long (en moyenne 130 μ) et comportant un ovaire avec des ovocytes sur un seul rang, sauf dans la zone de multiplication, une spermathèque volumineuse (10 μ de diamètre en moyenne) à paroi épaisse et remplie de spermatozoïdes sphériques de 1 μ du diamètre environ. Uterus bien développé, d'abord formé de cellules globuleuses puis comportant une chambre musculeuse. Tractus génital postérieur régressé, non fonctionnel, de 41-53 μ de long, composé d'une file de petites cellules dégénérées, dans laquelle on ne peut distinguer aucune des parties composantes. Aucun oeuf observé.

Cuticule marquée par des anneaux transversaux de 1,6 μ de large en moyenne, au milieu du corps; champ latéral formé de 4 lignes longitudinales équidistantes et occupant un quart du diamètre du corps au milieu de celui-ci. Les lignes extérieures sont légèrement crénelées et les lignes intérieures droites. Le champ latéral débute très en avant par 2 incisures aréolées; les deux lignes intérieures apparaissent au niveau de l'anneau nerveux où cesse l'aréolation; et les quatre lignes se prolongent presque jusqu'à l'extrêmité de la queue; le champ latéral est légèrement élargi et plus ou moins irrégulier au niveau de la vulve.

Queue longue de 8-12 μ (0,8-1 largeur anale), arrondie plus ou moins régulièrement; annélation continue à l'extrêmité avec parfois des irrégularités. Nombre d'anneaux de l'anus à l'extrêmité: 8 à 10. Phasmides très petites, situées au niveau de l'anus entre les deux incisures médianes du champs latéral.

Mâles: Aspect plus élancé que celui des femelles. Habitus en C très largement ouvert ou rectiligne.

Lèvres hémisphériques, comportant 4 anneaux, non séparées du corps. Stylet plus court que celui de la femelle (20-21 μ) mais de forme identique. Oesophage identique à celui de la femelle. Pore excréteur situé à 67-69 μ de l'avant. Cuticule, champ latéral et hémizonide identiques à ceux de la femelle.

Testicule unique, droit. Spicules longs de 14,5-15 μ, très faiblement arqués, robustes, larges à la partie distale, s'amincissant ensuite assez régulièrement et terminés par une pointe présentant un léger décrochement antérieur. Gubernaculum petit (4,5-5 μ) en forme de lame mince et légèrement courbe.

Queue pointue; bourse enveloppant toute la queue et débutant à environ une longueur de queue en avant de l'anus. Phasmides ponctiformes situées un peu en avant de l'anus.

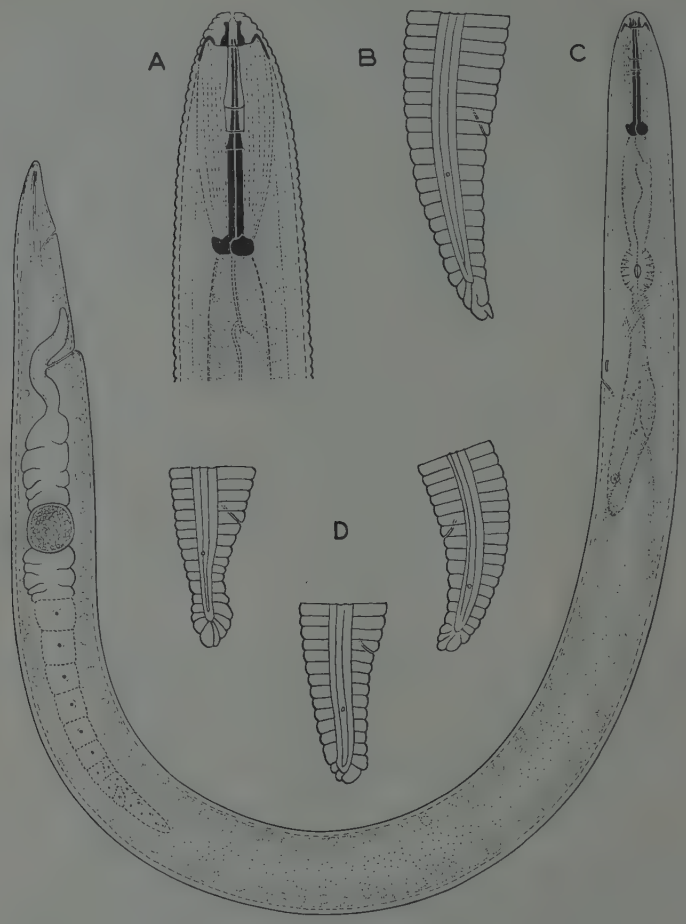
Diagnose: d'allure générale très semblable à *R. brevis* Whitehead, 1958, *R. intermedius* n. sp. s'en différencie par sa vulve située plus antérieurement (V = 80 % en moyenne, contre 90 %) et sa gonade femelle postérieure beaucoup moins régressée.

Hôte-type: racines de *Drypetes aylmeri* Hutch. & Dalz. (= *D. mottikoro* Leandri).

Lieu-type: Forêt de Yapo — Côte d'Ivoire.

Holotype: Femelle — lame 201-Ci-210 — Laboratoire de Nématologie I.D.E.R.T. — Abidjan — Côte d'Ivoire.

Allotype: Mâle — lame 199-Ci-210 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.



Pl. 2. — *Rotylenchoides variocaudatus* n. sp. — Femelle: A: tête. C: animal en entier. B: forme de queue la plus fréquente. D: différentes variations dans la forme de la queue. (gross.: C: 570 — A, B, D: 1150).

Rotylenchoides variocaudatus n. sp.

Dimensions: 10 ♀♀: L = 0,363-0,581 mm (moy. = 0,430 mm)
a = 19,3-27,5; b = 3,3-4,8; c = 22,7-33,6
V = $^{20,1-27,0}$ 86,3-88,9 % —

Holotype: ♀: L = 0,444 mm; a = 24,0; b = 4,0; c = 26,1
V = ^{26,1} 88,0 % —

Description:

Femelles: Lorsqu'elles sont tuées par la chaleur les femelles prennent une position variant de presque rectiligne à courbée en C très ouvert. Corps peu effilé vers l'avant, rétréci au niveau de la vulve et, plus rarement, à celui de l'anus.

Tête avec lèvres arrondies, non séparées du corps, comportant 4 anneaux, le troisième généralement plus large que les trois autres. Sclérotisation céphalique puissante; plaque basale s'étendant vers l'arrière sur trois anneaux du corps; sclérotisation interne des lèvres très apparente; guide du stylet bien développé. Stylet long de 26-31 μ (moyenne 28 μ), le protorhabdion et le telorhabdion étant de même longueur; boutons basaux arrondis à la partie distale et pourvus d'une courte pointe, plus ou moins émoussée, vers l'avant. Muscles protracteurs puissants.

Procorpus cylindrique, rétréci au niveau du bulbe médian; bulbe médian ovale; portion glandulaire basale recouvrant ventralement la partie antérieure de l'intestin. Orifice de la glande oesophagienne dorsale à 8-10 μ en arrière du stylet. Intestin sans caractères spéciaux.

Anneau nerveux situé immédiatement en arrière du bulbe oesophagien médian. Pore excréteur situé à 77-99 μ de l'avant, c'est-à-dire à mi-distance entre le centre du bulbe médian et l'extrémité postérieure de l'oesophage. Hémizonide plat, situé immédiatement avant le pore excréteur et s'étendant sur deux anneaux.

Tractus génital antérieur seul développé, long de 90-144 μ (moyenne 107 μ), comprenant un ovaire avec des ovocytes situés d'abord sur deux rangs puis sur un seul, une spermathèque globuleuse, à paroi épaisse, de 10-12 μ de diamètre, et remplie de spermatozoïdes sphériques de 1 μ environ de diamètre; utérus à paroi fine. Vulve en fente très profonde, atteignant des $^2/_3$ aux $^3/_4$ du diamètre correspondant, bordée de parois sclérotisées épaisses. Ovaire postérieur réduit à un sac post-utérin de 11-15 μ de long, terminé en pointe et accolé à la face ventrale. Oeufs non observés.

Cuticule ornée d'anneaux de 1,5 μ de large en moyenne au milieu du corps. Champ latéral occupant $^1/_5$ du diamètre du corps et composé de quatre lignes parallèles, les deux centrales droites, les deux extérieures très légèrement crénelées. Le champ latéral est quelque peu irrégulier au niveau de la vulve; les quatre lignes se prolongent généralement presque jusqu'à l'extrémité de la queue; plus rarement les deux lignes centrales fusionnent mais très postérieurement.

Queue de longueur variable: 14 à 24 μ (1,8 à 2,6 largeurs anales); forme variable également, généralement conique, avec des anneaux un peu plus larges que ceux du corps (1,9 μ); extrémité arrondie, anneaux irréguliers et grossiers; on ne distingue jamais à l'extrémité caudale de petites incisures supplémentaires; les phasmides ponctiformes sont situées aux $^2/_5$ antérieurs ou à la moitié de la queue, entre les deux lignes centrales du champ latéral, plus rarement sur l'une d'entre elles.

Mâles: inconnus.

Diagnose: *R. variocaudatus* n. sp. se différencie aisément de *R. brevis* White-

head, 1958 et *R. intermedius* n. sp. par sa queue non hémisphérique mais conique-allongée. Ce dernier caractère lui est commun avec *R. affinis* n. sp.; les différences entre ces deux espèces seront précisées dans la diagnose de *R. affinis* n. sp.

Lieu type: Sol; voisinage des racines de *Piper nigrum* L. et *Albizzia* sp. — Plantion SALCI — Ono — Côte d'Ivoire.

Holotype: Femelle — Lame 329-Ci-212 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire —

Deux femelles de *R. variocaudatus* étaient parasitées extérieurement et une autre intérieurement par une Microsporidie que nous rapportons à *Dubosqia penetrans* Thorne, 1940. Les spores sont un peu plus grandes que celles observées assez fréquemment en Côte d'Ivoire sur *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans-Stekhoven, 1941 et sur *Helicotylenchus* sp. (diamètre 4,5-5,5 μ contre 4-4,5 μ). Il n'est pas impossible que des races spécialisées existent à l'intérieur de ce parasite des nématodes.

Rotylenchoides affinis n. sp.

Dimensions: 10 ♀♀: L = 0,372-0,456 mm (moy. = 0,408 mm)
a = 19,5-25,2; b = 3,5-4,4; c = 26,4-35,6
V = $^{13,2\text{-}27,8}$ 82,5-87,8 % —

Holotype: ♀: L = 0,429 mm; a = 19,5; b = 4,4; c = 26,4
V = $^{20.0}$ 87,2 % —

Description:

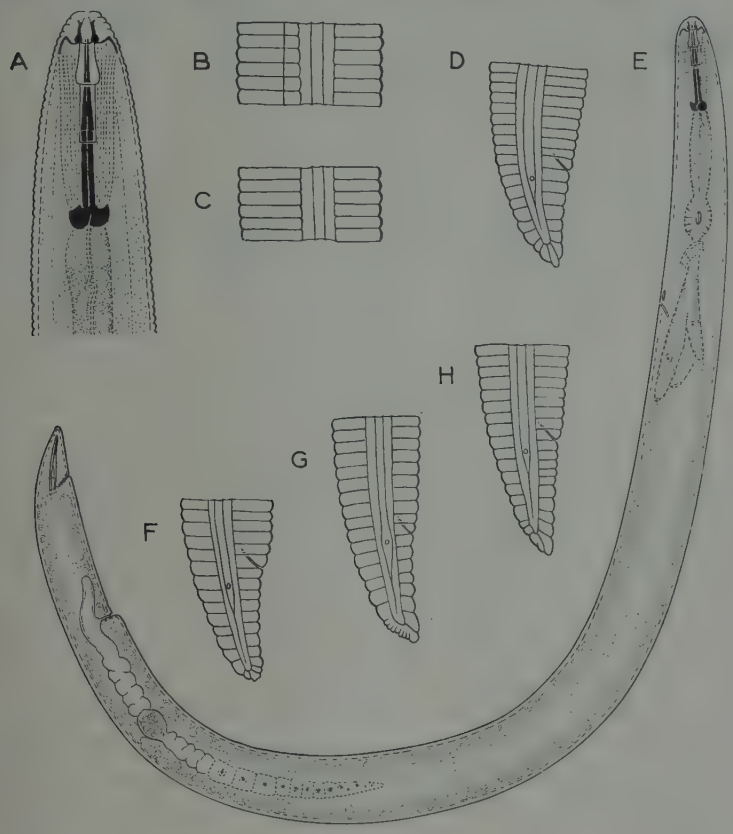
Femelles: Habitus très variable, rectiligne ou en C très ouvert, mais jamais spiralé. Corps peu effilé vers l'avant, marqué d'une incisure au niveau de la vulve et, moins fréquemment, à celui de l'anus.

Lèvres hémisphériques, non séparées du corps, comportant vraisemblablement quatre anneaux mais difficiles à percevoir car très peu en relief. Sclérotisation céphalique très développée; plaque basale débordant largement vers l'arrière; sclérotisation interne des lèvres et guide du stylet très apparents. Stylet long de 25-26,5 μ (moyenne 26 μ); protorhabdion et telorhabdion de même longueur; boutons basaux arrondis à la partie distale et pourvus d'une pointe vers l'avant. Muscles protracteurs du stylet puissants.

Procorpus cylindrique, légèrement rétréci au niveau du bulbe médian; celui-ci ovale; portion glandulaire basale trilobée, les trois lobes fréquemment légèrement détachés les uns des autres, le ventral s'étendant le plus en arrière. Orifice de la glande oesophagienne dorsale à 7-9 μ en arrière du stylet. Intestin sans caractères spéciaux.

Anneau nerveux large situé immédiatement sous le bulbe oesophagien médian. Pore excréteur situé à 68-85 μ de l'avant (moyenne: 77 μ). Hémizonide plat, situé immédiatement en avant du pore excréteur et s'étendant sur 2-3 anneaux.

Tractus génital antérieur seul développé, long de 58-104 μ (moyenne 86 μ), comprenant un ovaire droit avec ovocytes sur un seul rang sauf dans la partie proximale, une spermathèque sphérique à paroi épaisse, de 8 μ de diamètre en moyenne, remplie de petits spermatozoïdes globuleux de 1 μ environ de diamètre,



Pl. 3. — *Rotylenchoides affinis* n. sp. — Femelle: A: tête. E: animal en entier. B: champ latéral aberrant. C: champ latéral normal. D, F, G, H: différentes variations dans la forme de la queue. (gross.: E: 570 — A, B, C, D, F, G, H: 1150).

et un utérus à paroi mince, tourmentée. Vulve en fente profonde. Gonade postérieure réduite à un sac post-utérin de longueur variable (8-23 μ). Oeufs non observés.

Cuticule ornée d'anneaux de 1,3 μ de large en moyenne au milieu du corps. Champs latéral occupant environ $^1/_5$ du diamètre du corps et composé de quatre lignes parallèles, droites. Sur certains individus il existe une cinquième ligne supplémentaire située ventralement donnant au champ latéral un aspect très particulier (Pl. 3, fig. B).

Queue de longueur variable (11,5-16 μ soit 1,3-1,7 largeurs anales), conique - allongée, à extrémité plus ou moins pointue. Phasmides au niveau de l'anus ou très peu postérieurement, situées entre les deux lignes centrales du champ latéral ou parfois sur une de ces deux lignes. Champ latéral se prolongeant presque jusqu'à l'extrémité de la queue; les lignes centrales fusionnent en une seule ligne à hauteur de la moitié de la queue environ. Anneaux de l'extrémité caudale irréguliers, le plus souvent plus larges que la moyenne des anneaux du corps; sur certains individus de petites indentations supplémentaires existent à l'extrémité de la queue qui est alors non terminée en pointe mais plus ou moins tronquée.

Mâles: inconnus.

Diagnose: *R. affinis* n. sp. se différencie aisément de *R. brevis* Whitehead, 1958 et de *R. intermedius* n. sp. par la forme de sa queue, conique - allongée et non hémisphérique. Cette espèce se différencie beaucoup plus difficilement de *R. variocaudatus* n. sp. La portion antérieure du corps est remarquablement semblable chez les deux espèces, tout au plus peut-on noter un stylet un peu plus long chez *R. variocaudatus* n. sp. (26-31 μ, contre 25-26,5 μ). Les caractères servant à différencier ces deux espèces sont ceux de la queue; chez *R. variocaudatus* n. sp. la queue est plus allongée (longueur = 1,8-2,6 largeurs anales contre 1,3-1,7) et les phasmides y sont situées non au voisinage de l'anus ou très peu postérieurement comme chez *R. affinis* n. sp. mais aux $^2/_5$ antérieurs ou à la moitié; de plus chez *R. variocaudatus* n. sp. les deux lignes centrales du champ latéral se poursuivent distinctes l'une de l'autre jusqu'à l'extrémité de la queue ou ne se rejoignent que très postérieurement alors que chez *R. affinis* n. sp. les deux lignes centrales fusionnent en une seule au niveau du milieu de la queue environ. Un autre caractère pouvant servir à différencier ces deux espèces consiste en la position de la vulve et de l'anus l'une par rapport à l'autre: si l'on mesure la distance vulve-anus en longueurs caudales on trouve chez *R. variocaudatus* n. sp. des chiffres variant de 1,6 à 2,2 avec une moyenne de 2 et chez *R. affinis* n. sp. des chiffres de 2,4 à 4,4 avec une moyenne de 3,1; de telles mesures effectuées sur 10 femelles de chaque espèce ont montré que les différences observées étaient statistiquement significatives avec une probabilité supérieure à 99 %.

Lieu type: Sol; voisinage des racines de *Coffea* sp. Route entre Man et Touba (Côte d'Ivoire). Leg. G. de Guiran.

Holotype: Femelle — Lame 420-Ci-257 — Laboratoire de Nématologie — I.D.E.R.T. — Abidjan — Côte d'Ivoire.

SUMMARY

Three new species of the genus Rotylenchoides *Whitehead, 1958* (*Nematoda-Tylenchida*)

The author describes and figures three new species belonging to the genus *Rotylenchoides* Whitehead, 1958: *R. intermedius* n. sp., *R. variocaudatus* n. sp. and *R. affinis* n. sp. The first, very close to the type species *R. brevis* Whitehead, 1958, differs by having a posterior ovary which is not reduced to a post-uterine sac but to a rather long, single file of degenerated cells, and by having a vulva more anteriorly placed. *R. variocaudatus* n. sp. and *R. affinis* n. sp. differ from the two other

species by having a tail not hemispherical but longer than the anal diameter and generally conoid. These two species differ from each other in the location of the phasmids and the structure of the lateral field on the tail. Males are known for *R. intermedius* n. sp. but not for the other two new species. The author amends the diagnosis of the genus *Rotylenchoides:* the posterior ovary is not functional but reduced to a rather long single file of degenerated cells or to a post-uterine sac; the reference to the shape of the tail is suppressed because of its variability. The taxonomic position of the genus *Rotylenchoides* seems to be in the subfamily Hoplolaiminae, near *Helicotylenchus* Steiner, 1945, notwithstanding the reduction of the posterior ovary. This single last character does not seem sufficient to justify the existence of the subfamily Rotylenchoidinae Whitehead, 1958.

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Nematologica 5 (1960): 18-26. E. J. Brill, Leiden

DIE RÄUMLICHE VERTEILUNG DES PRIMÄRBEFALLS VON *HETERODERA SCHACHTII* SCHMIDT IN DEN WIRTSWURZELN

VON

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Bei der Durchsicht von mit Rübennematoden befallenen Wirtswurzeln in Hinblick auf die zeitliche Folge des Infektionsganges (APEL & KÄMPFE, 1957) war aufgefallen, daß auch die räumliche Verteilung der eingewanderten Larven und ihrer weiteren Entwicklungsstadien in der Wurzel nicht homogen zu sein scheint. Aus Kulturversuchen in künstlichen Medien ist bekannt, daß die Abschnitte unmittelbar oberhalb der Wurzelspitzen eine Anlockwirkung auf die wandernden Larven ausüben, wie dies die Abbildungen von WIDDOWSON, DONCASTER & FENWICK (1958) für den Kartoffelnematoden zeigen. Für das Wurzelgallenälchen (*Meloidogyne hapla* Chitwood) konnte WIESER (1955) ebenfalls eine Attraktivzone im Bereich des Streckungsabschnittes der jungen Wirtswurzeln nachweisen. Auch WALLACE (1958a) nimmt eine Anlockwirkung für *Heterodera*-Larven an, die von der Wurzel ausgeht, wobei die Larven sich gegen das Konzentrationsgefälle des Attraktivstoffes orientieren. Aber auch die Ursprungsstellen von auswachsenden Seitenwurzeln 1. und höherer Ordnung sind Orte größerer Larvendichte im Wurzelsystem, wie WIDDOWSON, DONCASTER & FENWICK (1958) erwähnen. Ein gleicher Befund läßt sich den Abbildungen der Arbeit von BERGMAN (1958) entnehmen. Hingegen meint DICKINSON (1959), daß die Anheftung von *Heterodera schachtii* an der Wurzel auf rein physikalischen Ursachen beruhen könnte. Die Eignung einer Wurzel als Wirtsmedium würde sich erst beim Anstechen der Wurzelzellen herausstellen. Einen ähnlichen Gedankengang verfolgt KÜHN (1959), der annimmt, daß das Finden der Wirtswurzel durch den Kartoffelnematoden auch ohne die Annahme eines Reizgefälles nur auf Grund des Zufalles möglich sein soll.

In den folgenden Versuchen ist die Verteilung des Primärbefalls für *Heterodera schachtii* in natürlich verseuchter Erde geprüft worden, wobei intakte und vollständige Wurzelsysteme den Larven bei der Einwanderung zur Verfügung standen.

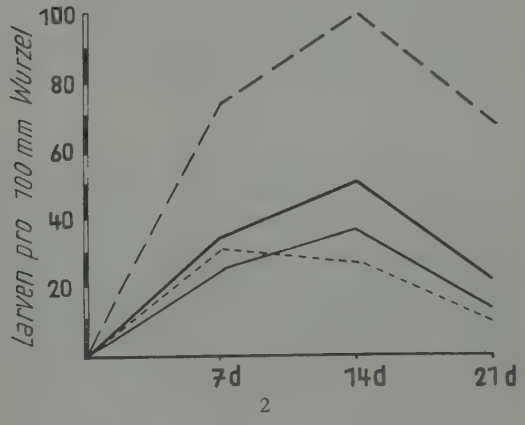
METHODE

Als Wirtspflanzen wurden Raps (*Brassica napus* L. v. *arvensis* (Lam.) Thell.), in Vergleichsversuchen auch Zuckerrübe (*Beta vulgaris* L. f. *altissima* D.C.) her-

angezogen und dazu in poröse Blumentöpfe je 40 Samen eingebracht. Die Erde zeigte eine Verseuchung von ca. 40 lebenden Eiern bzw. Larven pro 1 ccm lufttrockener Erde. Alle Töpfe erhielten gleichmäßig viel Feuchtigkeit und standen unter gleichen Lichtverhältnissen bei + 20° C. Die Durchwurzelung der Töpfe war gut. Nach 7, 14 und 21, für Zuckerrüben auch nach 28 Tagen erfolgten die Entnahmen der Wurzeln, wobei pro Topf 10 gut entwickelte Pflanzen herangezogen wurden. Die gereinigten Wurzeln wurden mit Jodjodkalium- bzw. Osmiumtetroxyd-Lösung (0.1 %) angefärbt und die eingewanderten Larven ausgezählt. Anschließend war die Zahl der Seitenwurzeln festzustellen und die Wurzel zu vermessen.

Bei der Auszählung der eingewanderten Stadien ist unterschieden in solche, die in den Bereich der Wurzelspitze (SP), solche, die in den Bereich der Ursprungsstelle einer Nebenwurzel (NW) und schließlich solche, die in die übrigen Strecken des Wurzelsystems (W) eingewandert waren. Da erfahrungsgemäß die eigentliche Wurzelspitze selbst nicht befallen wird, sondern erst die sich basiswärts anschließende Streckungszone, zeigen die Spitzen sehr junger Nebenwurzeln keinen Befall. Es sind deshalb nur die über 3 mm langen Seitenwurzeln für die Feststellung des Spitzenbbefalls herangezogen worden. Der Einwanderungsbereich der Wurzelspitze ist für die Nebenwurzeln mit 2 mm, für die Hauptwurzel mit 3 mm Länge angesetzt worden. Ein um die Ursprungsstellen der Seitenwurzeln gedachter Kreis von 1 mm Durchmesser schließt die Larven ein,

1



2

Abb. 1: Verteilung der einzelnen Wurzelabschnitte (schematisch). Kreise = Nebenwurzel-Ursprungsstellen (NW), schwarze Bereiche = Spitzenabschnitte (SP), nicht gekennzeichnete Bereiche = übrige Wurzelstrecken (W).

Abb. 2: Befallsdichte in den einzelnen Wurzelbereichen für Raps. — = Gesamtbefall pro Wurzel: — — = Nebenwurzel-Ursprungsstellen (NW); - - - - = Spitzenbereiche (SP); —— = sonstige Wurzelbereiche (W).

die im Bereich der Seitenwurzeln eingewandert sind. Dabei wurden auch die Larven mitgezählt, deren größerer Körperabschnitt noch im Kreisbereich lag oder deren Kopfende in diesen hineinragte (Abb. 1).

Des weiteren wurde bei der Auszählung der eingewanderten Larven unterschieden, ob diese einzeln oder in Gruppen räumlich eng beieinander lagen. Schließlich wurde registriert, wie hoch der Anteil neu zugewanderter Larven II bei den Kontrollauszählungen nach 14 und 21 Tagen war und ob diese Neuzugänge wieder die Nähe schon eingewanderter Tiere suchten. Für Gruppenbildungen galt als Maß des noch zulässigen Abstandes die normale Körperlänge einer Larve. Wurde dieser Abstand überschritten, so lag eine Gruppenbildung nicht vor. In den Hauptversuchen belief sich die Zahl der untersuchten Wurzelsysteme für jeden Kontrolltermin auf 90 bis 100 Stück.

ERGEBNISSE

Zunächst sei die Verteilung der Larven in den Rapswurzeln betrachtet. Schon bei der Durchsicht der Wurzeln auf ihren Befall entsteht der Eindruck, daß die Bereiche, an denen Nebenwurzeln abzweigen, häufiger und dichter befallen sind als die übrigen Wurzelstrecken, in denen oft über längere Abschnitte kaum Larven zu verzeichnen sind. Vergleicht man aber den absoluten Befall der NW-Bereiche mit dem der übrigen Wurzelstrecken (also Hauptwurzel + Nebenwurzel einschließlich Spitzen, aber minus Ursprungsstellen aller Seitenwurzeln erster und höherer Ordnung), so ergeben sich kaum nennenswerte Unterschiede:

	Wurzel (W)	*NW-Bereiche*
7 d	19,6	12,4 Larven pro Pflanze; n = 2884 Larven
14 d	31,2	30,4 „ „ „ n = 5854 „
21 d	23,0	24,8 „ „ „ n = 4023 „

Jedoch führt ein solcher Vergleich der absoluten Befallszahlen zu einem falschen Bild, denn die genannten Zahlen beziehen sich nicht auf vergleichbare Wurzelstrecken. Berechnet man pro Wurzelsystem den Streckenanteil, der auf die NW-Bereiche entfällt, so ergibt sich, daß dieser etwa nur $^1/_6$ bis $^1/_5$ der Gesamtlänge des Wurzelsystems ausmacht. Die Befallsdichte (= Zahl der Larven pro 100 mm Wurzelstrecke) zeigt unter Zugrundelegung der entsprechenden tatsächlichen Wurzelanteile nun folgendes Bild:

	7 d	14 d	21 d	
Befallsdichte Gesamtwurzel	33,6	50,1	21,4	Larven pro 100 mm Wurzel
Befallsdichte NW-Bereiche	73,0	99,7	68,8	„ „ „ „ „
Befallsdichte übriger W-Bereich	25,2	33,8	12,6	„ „ „ „ „

Zur Berechnung der NW-Bereiche ist für jede Seitenwurzel-Ursprungsstelle 1 mm veranschlagt worden. Da bisweilen 2 Seitenwurzeln in einer Höhe entspringen, ist in solchen Fällen nur einmal 1 mm angesetzt worden. Dies trifft je nach Alter der Pflanzen und nach Zahl der Seitenwurzeln für 5 bis 12 % aller Seitenwurzeln zu.

Nach 7 Tagen waren durchschnittlich 17,7, nach 14 Tagen 33,1 und nach 21 Tagen 40,5 Seitenwurzeln pro Pflanze sichtbar. Dabei sind auch solche mitgezählt, die eben im Begriff waren, nach außen durchzubrechen. Diese sehr jungen Seitenwurzeln zeigen jedoch nicht immer Befall. Die Nichtbewertung solcher junger Seitenwurzeln würde die Befallsdichte noch stärker zu Gunsten der NW-Bereiche verschieben.

Die Tabelle zeigt eindeutig eine höhere Larvendichte für die Ursprungsstellen der Seitenwurzeln im Vergleich zu den übrigen Wurzelstrecken. Die Differenz gegenüber einem errechneten Sollwert ist statistisch hoch gesichert. Damit erweist es sich, daß für die in die Wurzel einwandernden Larven die NW-Bereiche bevorzugte Invasionsgebiete sind und daß der Befall der übrigen Wurzelstrecken in weit geringerem Maße erfolgt. Auch in einem vorausgegangenen Versuch, bei dem für jeden Kontrolltermin wenigstens 250 Wurzelsysteme untersucht worden waren, hatte sich ein gleiches Bild ergeben.

Nicht den Erwartungen entsprach jedoch der Befall, der sich für die Wurzelspitzenabschnitte (2 bzw. 3 mm lang oberhalb des eigentlichen Wurzelapex) ergeben sollte. Bei weitem nicht alle Wurzelspitzen, besonders nicht die jüngeren, waren befallen. Wurzeln unter 3 mm Länge sind überhaupt nicht berücksichtigt worden. Nach 7 Tagen sind in den Wurzelspitzen einer Pflanze im Durchschnitt nur 2,7 Larven zu finden, nach 14 Tagen sind es 7,9 und nach 21 Tagen 4,5 Larven. Berechnet man wieder die Befallsdichte für die Spitzenanteile, so ergeben sich folgende Werte:

7 d = 28,2 Larven pro 100 mm Spitzenbereich
14 d = 25,3 „ „ „ „ „
21 d = 8,3 „ „ „ „ „

Der Durchschnittswert gibt jedoch kein reales Bild von der tatsächlichen Verteilung der Larven in den Spitzenbereichen. Ein großer Teil der Spitzen zeigte überhaupt keinen Befall, andere Spitzenabschnitte waren wiederum von mehreren Larven gleichzeitig besiedelt worden. Es scheint diese Beobachtung eine Parallele zu Bemerkungen von WIDDOWSON, DONCASTER & FENWICK (1958) darzustellen, die in ihren Agarkulturen ebenfalls eine deutliche Bevorzugung bestimmter Wurzelspitzen feststellten, während andere offenbar völlig gemieden wurden. Praktisch sämtliche in den Wurzelspitzen gefundenen Individuen sind Larven II, nur in älteren Spitzenbereichen treten auch Larven III in geringer Zahl auf. Auf diese Tatsache wird später noch einmal einzugehen sein.

Betrachtet man die Befallsdichten für die gesamte Wurzel und den Befall der einzelnen drei unterschiedenen Wurzelabschnitte für die hier gewählten Kontroll-

termine (Abb. 2), so ergibt sich ein Befallsmaximum nach 14 Tagen, während nach 21 Tagen durch das verstärkte Wurzelwachstum die Besiedlungsdichte bereits absinkt. Die durchschnittliche Wurzellänge betrug nach 7 Tagen 95 und nach 14 Tagen 123 mm, nach 21 Tagen aber 223 mm. Ein weiterer Grund für die Verminderung der Befallsdichte nach 21 Tagen liegt in dem zu diesem Zeitpunkt bereits beginnenden Auswandern reifer Männchen, vor allem aber in einem auffälligen Rückgang der Zahl neu einwandernder Larven II. Nach 7 Tagen gehören etwa 40 % aller vorhandenen Larven dem Stadium II an, der Rest wird fast ausschließlich aus Larven III gebildet. Nach 14 Tagen beträgt der Anteil der Larven II in den Wurzeln nur noch 18,7 %, nach 21 Tagen sogar nur 7,9 %. Auf 100 mm Wurzellänge umgerechnet wird der Rückgang im Neubefall noch deutlicher:

7 d = 13,5 L II pro 100 mm Gesamtwurzellänge
14 d = 9,4 „ „ „ „ „
21 d = 1,8 „ „ „ „ „

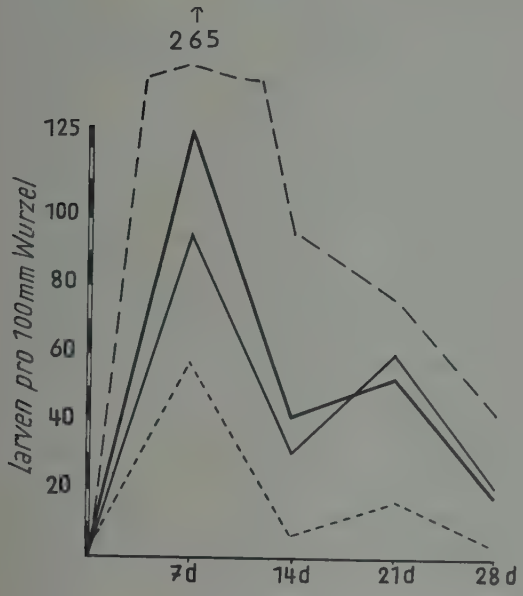
Dieser Rückgang in der Neueinwanderung von Larven II fällt umso mehr auf, als die Wurzelmasse bis zum 21. Tage erheblich zunimmt und damit neue Einwanderungsmöglichkeiten gegeben wären. Der überwiegende Teil der nach 14 und 21 Tagen festgestellten Larven II ist in die Spitzenbereiche der Nebenwurzeln eingewandert, ein geringerer Teil hat die Ursprungsstellen von Nebenwurzeln befallen. Neueinwanderungen in die Haupt- und älteren Nebenwurzelstrecken finden praktisch überhaupt nicht statt. Damit erklärt sich auch der verhältnismäßig hohe absolute Befall der Spitzenregion nach 14 Tagen mit 7,9 Larven pro Pflanze, denn zur gleichen Zeit hat sich die Anzahl der Seitenwurzeln gegenüber 7 Tagen fast um das Doppelte erhöht.

Für die einzelnen Befallsbezirke innerhalb der Wurzel ist noch eine Differenzierung in einzeln und in Gruppen zusammenliegende Larven erfolgt. Allgemein kann festgestellt werden, daß mit zunehmender Befallsdichte in den Wurzeln der Prozentsatz der in Gruppen zusammenliegenden Larven steigt. Er ist mit 35,5 % aller Larven demzufolge bei der Kontrolle nach 14 Tagen am höchsten. Das bedeutet, daß zu diesem Zeitpunkt etwa jede 3. Larve in unmittelbarer Nähe einer anderen zu finden ist.

Die gleiche Tendenz ist auch für die Verhältnisse in den einzelnen Wurzelbereichen zu erkennen. Besonders nach 14 Tagen liegt die Gruppenbildung in den NW-Bereichen mit 42,6 % aller Larven auffallend hoch. Sie erreicht damit zugleich den Höchstwert für den gesamten Versuchsablauf. Zur gleichen Zeit tritt für diesen Wurzelbereich auch die höchste überhaupt erreichte Befallsdichte auf (vergl. Abb. 2). Ähnlich liegen die Verhältnisse auch nach 21 Tagen.

Für die nach 14 und 21 Tagen neu einwandernden Larven II kann gezeigt werden, daß diese nur sehr selten in der unmittelbaren Nähe älterer, schon früher in die Wurzeln eingedrungener Larven zu finden sind. Die Neueinwanderung erfolgt fast ausschließlich in noch kaum befallene Wurzelspitzen junger Seiten-

wurzeln, die etwa 70 bis 90 % des Neubefalls an Larven II aufnehmen. Der Rest verteilt sich auf die Seitenwurzelbereiche außerhalb der Wurzelspitzen und auf die Ursprungsstellen junger Seitenwurzeln. Gruppenbildungen von später eingewanderten Larven II treten praktisch nur in den Wurzelspitzenbereichen auf.



3

4

Abb. 3: Befallsdichte in den einzelnen Wurzelbereichen für Zuckerrübe. Legende wie Abb. 2.
Abb. 4: Rapswurzel, 21 Tage alt, mit eingezeichneten Befallsorten (Punkte).

Ein Vergleich der bisher gewonnenen Befunde mit denen, die für Zuckerrüben gefunden worden sind, ergibt in allen wesentlichen Punkten Übereinstimmung. Die Befallsdichten für die einzelnen unterschiedenen Wurzelbereiche sind in Abb. 3 dargestellt. Auffallend ist hier ein besonders hoher Erstbefall nach 7 Tagen. Auch für Zuckerrüben stellen die Nebenwurzel-Ursprungsstellen die bevorzugten Befallsgebiete dar, während die Wurzelspitzen eine Befallsdichte zeigen, die ständig unter dem Wert für das gesamte Wurzelsystem liegt. Da junge Zuckerrübenpflanzen nicht in dem Maße Nebenwurzeln bilden wie der Raps, liegt der Befall für den übrigen Wurzelbereich (W) etwas höher als bei den Rapswurzeln. Die Neigung zur Gruppenbildung ist in den Zuckerrübenwurzeln noch stärker ausgeprägt als in den Rapswurzeln. Besonders in den Nebenwurzel-Bereichen (NW) ist sie wiederum besonders häufig. In Zuckerrüben hält die Neuinfektion mit Larven II ebenfalls nicht mit dem Zuwachs an Wurzelmasse Schritt. Die Einwanderungszahlen gehen vielmehr allmählich zurück. Auch hier

ist eine klare Bevorzugung noch nicht befallener Wurzelteile durch die neu einwandernden Larven II festzustellen (73 % der Larven sind in noch nicht befallenen Wurzelteilen zu finden).

DISKUSSION DER BEFUNDE

Betrachtet man das Gesamtbild, das sich für den Ablauf und die Verteilung des Primärbefalls in den ersten 3 bzw. 4 Wochen für die beiden hier untersuchten Wirtspflanzen ergibt, so kann festgestellt werden, daß eine deutliche Abhängigkeit des Befalls von der Wurzelausbildung besteht. Die Unterschiede zwischen den Ergebnissen in Agarkulturen und den hier geschilderten Topfversuchen liegen in erster Linie in der hier an über 1000 Wurzelsystemen nachgewiesenen geringen Befallsdichte der Spitzenbereiche. Ein wichtiger Grund für diese Abweichung scheint mir darin zu liegen, daß im Erdreich völlig andere, im ganzen sicher ausgeglichenere Konzentrations- und Reizgefälle gegeben sind als in Agarkulturen. In diesen wirken außerdem nur wenige, sehr junge Wurzeln und daher vor allem solche mit relativ großen Spitzenanteilen auf ein sonst weitgehend indifferentes Medium ein. Außerdem darf wohl angenommen werden, daß diese Wurzelteile in Agar stoffwechselphysiologisch nicht als Norm angesehen werden können. Hinzu kommt, daß in Versuchen mit Agarkulturen mit hohen Ei- bzw. Larvenmengen gearbeitet wird, denen zudem die relativ geringe Wurzelmasse in unmittelbarer Nähe zur Verfügung steht. Derartige Agarversuche müssen daher wohl als Modellfälle betrachtet werden, deren Ergebnisse extrem stark akzentuiert sind. Unter Bodenbedingungen überlagern sich die Reizgradienten — soweit solche wirksam sind — zweifellos viel stärker. Schließlich wissen wir auch noch nichts Endgültiges über die Entfernungen, über die Wurzelreize auf den Zysteninhalt und die wandernden Larven wirksam sind (WALLACE, 1958a, nimmt für den Schlüpfreiz etwa 4,5 cm an, WEISCHER, 1959, 1-2 cm).

Auf Grund der vorliegenden Befunde scheint der spezielle Einwanderungsreiz erst auf kürzeste Entfernung wirksam zu werden. Dabei bieten sich als günstige Einfallstore die Spitzenbereiche und vor allem die Entstehungsorte von Seitenwurzeln an. Beide können sowohl reizphysiologisch als auch rein mechanisch die Einwanderung fördern. Sehr junge Wurzeln erlauben jedoch die Invasion auch außerhalb der genannten Bereiche.

Schließlich ist es wichtig zu wissen, ob und wie weit eine in die Wurzel eingedrungene Larve im Wurzelgewebe aktiv wandert. Dadurch wäre eine Auflockerung des Spitzenbefalls oder eine Massierung an den Seitenwurzeln möglich. Gegen größere aktive Wanderungen in den Wurzeln spricht aber, daß das jeweilige Entwicklungsalter der Larven immer gut mit dem Alter der Wurzelteile übereinstimmt und daß junge Larven auch unmittelbar an den Seitenwurzeln-Entstehungspunkten einwandern und dort bleiben. Durch die passive Verlagerung der Larven II infolge des Wurzelwachstums ist jedoch tatsächlich eine gewisse Auflockerung des Spitzenbefalls gegeben.

Erwähnenswert ist weiter die Beobachtung, daß sich die Weibchen vorwiegend

an den älteren Teilen der Hauptwurzeln finden, während Männchen schon sehr bald an Nebenwurzeln auftreten. Das liegt in der längeren Entwicklungsdauer der Weibchen begründet, während durch die schnellere Entwicklung der Männchen diese gleichzeitig auch an älteren Nebenwurzeln und jüngeren Hauptwurzelabschnitten zur Reife gelangen. Die Männchen sind wesentlich häufiger in Gruppen mit anderen Männchen oder mit etwas jüngeren Stadien zusammen anzutreffen, als dies bei Weibchen der Fall ist. Es fiel wiederholt auf, daß bei stärkeren Nematodenansammlungen an Seitenwurzel-Ursprungsstellen die Mehrzahl die männliche Entwicklungsrichtung einschlug oder schon als Männchen ausgebildet waren. Für Raps traten 55 % der Männchen in Gruppen auf, aber nur 13 % der Weibchen zeigten eine ähnliche Vergesellschaftung. Auch für Zuckerrüben überwiegen die gruppenbildenden Männchen deutlich. Das Gesamtverhältnis der Männchen zu den Weibchen war in solchen Fällen sehr zugunsten der Männchen verschoben. Es ist nicht ausgeschlossen, daß sich dabei die gleichen Faktoren auf engstem Raum auswirken, die ELLENBY (1954) für die Beeinflussung des Geschlechts für den Kartoffelnematoden angedeutet hat.

Die unterschiedlich starke Besiedlungsdichte der einzelnen Wurzelbezirke, die eine auffallende Bevorzugung der Seitenwurzel-Ursprungsstellen gezeigt hat, läßt sich mit der Annahme einer von den aktivsten Wurzelteilen ausgehenden unmittelbaren Reizwirkung, die über eine gewisse Distanz wirksam ist, erklären. Sie ist aber ebenso gut mit der Vorstellung vereinbar, daß die Larven auf zufälligem Wege in unmittelbare Wurzelnähe gelangen und dann durch Wandern an der Wurzel entlang die mechanisch und ernährungsphysiologisch günstigen Befallspunkte aufsuchen, um dort einzudringen. Vorläufig scheinen jedoch mehr Tatsachen für einen über den zufälligen Wurzelkontakt hinausgehenden „Fernreiz" bei der Einwanderungssteuerung der Larven zu sprechen. Dabei bleibt die ausgezeichnete Abstimmung des Befallsverlaufes mit der fortschreitenden Veränderung des jungen Wurzelsystems als interessante Tatsache bestehen. Eine noch weitergehende Analyse des Befalls wird hier weitere Aufschlüsse bringen können.

SUMMARY

The position in host roots of the primary attack by Heterodera schachtii *Schmidt*

Roots of rape (*Brassica napus* L. var. *arvensis* (Lam.) Thell.) and sugar beet (*Beta vulgaris* L. f. *altissima* D.C.) grown in pots inoculated with beet eelworm (*Heterodera schachtii* Schm.), were examined after 7, 14, 21 and 28 days to determine the spatial distribution of invading larvae, the root tips, points of origin of lateral roots and the intervening regions being considered separately. The total numbers of larvae present indicated no preference for any one of these regions. Reference to the numbers in lengths of roots from individual regions, however, shows a clear preference for the points of origin of the lateral roots at all the times of examination. In contrast to results from experiments in agar, the root tips show only a relatively small degree of infestation. This is probably due to the different conditions for infection in soil and in agar. The tendency of the larvae to lie together in compact groups was also studied. The greatest aggregations exist at the points of origin of lateral roots and are coupled with greater infection densities. After 14, 21 and 28 days newly invaded second-stage larvae are found almost entirely in the terminal regions of the lateral roots and also in the points of origin of lateral roots. Previously unattacked parts of roots are clearly preferred. Males are more numerous in the aggregations. The observed pattern of invasion is in accordance with the hypothesis of root attraction and also with that of random movement but favours the former.

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DIFFERENTIAL NUTRITIONAL REQUIREMENTS FOR REPRODUCTION OF TWO STRAINS OF *CAENORHABDITIS ELEGANS* IN AXENIC CULTURE

BY

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Caenorhabditis elegans (Maupas) has been maintained in axenic culture for more than three years. Two separate isolations, one in France and one in England, have been made and are here designated as *Bergerac* strain and *Bristol* strain respectively. Although morphologically similar [2]) and successfully crossed by Nigon (NICHOLAS, DOUGHERTY & HANSEN, 1959, p. 233), the two strains have shown different cultural requirements. This difference was first observed in 1958 (NICHOLAS *et al., ibid.*), using a supplemented chemically defined medium, and has now been investigated in a wider range of media.

MATERIALS AND METHODS

The Bergerac strain was derived from a single hermaphrodite collected by Professor Victor Nigon from garden soil near Bergerac. The Bristol strain was derived from an inoculum of approximately 50 worms collected by L. N. Staniland from compost of a commercial mushroom bed near Bristol. The present axenic cultures of both were established by Dr. W. L. Nicholas in 1956, using the method described by NICHOLAS & MCENTEGART (1957). Monoxenic cultures were later initiated by inoculating 0.25 ml of a suspension of the axenic culture on to a slant of nutrient agar on which *Escherichia coli* was growing.

The stock axenic cultures have been maintained largely in media derived from liver (see next paragraph) with an occasional use of media containing chick embryo extract. The cultures are grown at 17°C in screw-capped tubes containing approximately 1 ml of medium. For subculturing, the old culture, containing eggs and worms of all sizes, is shaken into suspension and approximately 0.1 ml is transferred to fresh medium. To maintain vigorous growth, transfers

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[2]) We are indebted to Dr. B. G. Chitwood for extending Professor Nigon's observations (see NICHOLAS *et al.*, 1959). The structure of the caudal alae, the number and position of the papillae, and the shape of spicules are identical in the rare males in our current cultures of the two strains.

are made at intervals of approximately three weeks; the cultures remain viable, however, for at least four months.

Liver medium is composed of a 1 : 1 mixture of an autoclaved liver preparation (ALE) and of a liver protein fraction precipitated by ammonium sulfate (LPF-C). The composition and preparation of these liver components and of the chick embryo extract (CEE) used have been described (NICHOLAS *et al.*, 1959, p. 219). Preparation of CEE has now been simplified by employing Seitz filtration for sterilization.

In addition to these fresh organic materials, a dehydrated liver extract [1]) has been used. A solution was made by adding 450 mg of the dry powder to 10 ml distilled water and autoclaving at 121° C for 10 minutes. A small amount of precipitate formed. The preparation was decanted, and only the clear supernatant liquid was used. The dry weight of this preparation was 32 mg per ml.

The tissue extracts are stored at —17° C in aliquots of approximately 30 ml. These are thawed prior to use. During repeated freezing and thawing a precipitate forms; this is dispersed before withdrawing the required aliquot. Some loss of activity occurs, which can be neglected in stock cultures. Since each preparation of tissue extract differs, a check is made of the dry weight, protein (LOWRY, ROSEBROUGH, FARR & RANDALL, 1951) and nitrogen content (Table I). It is

TABLE I

Tissue extracts used in media for C. elegans *analyzed after Seitz filtration*

Extract	Dry wt. mg/ml	Nitrogen mg/ml	Protein (Lowry) mg/ml
Autoclaved liver extract, ALE	45	3.49	18.1
Liver protein, fraction C, LPF-C	73	6.09	40.3
Chick embryo extract, CEE	22	1.65	7.5

fully appreciated that such determinations by no means characterize the extract; however, they can serve as a guide to the evaluation of individual preparations.

Chemically defined media, GS and GM, have been designed to supply the nutritional requirements of a related species, *C. briggsae*. Basal media of the GS-type consist of amino acids, vitamins, glucose, and salts; GM-type media are more complex in containing additional amino acids and vitamins as well as nucleotides and other nutrients (DOUGHERTY, HANSEN, NICHOLAS, MOLLETT & YARWOOD, 1959, p. 179).

Monoxenic cultures are carried on nutrient agar slants; subcultures are made by transferring a small lump of agar containing both worms and bacteria to a fresh sterile slant. Monoxenic cultures have also been carried on a less rich agar prepared according to the formula of NIGON (1949).

The cultures consist almost entirely of hermaphrodites, with males occurring

[1]) Oxoid Co., London, E.C. 4, England.

infrequently. With the Bristol strain, no males were found among 1817 adults from the stock axenic cultures; two males were found in 2434 adults from monoxenic cultures, and one male among 98 adults in the individual tube tests. In the Bergerac strain three males have been found among 171 adults in the individual tube tests, and one male and two males respectively among a large number of adults examined in the axenic and monoxenic stock cultures. In both strains, "abnormal" males, in which the structures of the bursa were not fully developed, were rather more frequent.

For the tests described here, the rate of growth at 20° C of individual larvae was observed. Adults from stock axenic cultures were washed free of medium by transfer through several dishes of M/15 phosphate buffer, pH 7, and then left for 24 hours to lay eggs in buffer. The newly hatched larvae were then inoculated singly into the test medium, distributed in aliquots of 0.25 ml in small tubes, 10 mm in diameter. These methods, designed in this laboratory for precise growth studies (DOUGHERTY *et al.*, 1959, p. 192), allow a high degree of reproducibility. The rate of growth of these larvae was observed, the time to mature and produce offspring noted (see Generation time, Table II), and the resulting cultures followed for at least 20 days.

RESULTS

The maturation rate of larvae in various media is shown in Table II. Larvae of Bristol strain grew faster than those of Bergerac in each of the different media and gave rise to appreciably larger populations; in the best organic medium (C, Table II) populations of at least 5,000 developed in 22 days from the original hermaphroditic Bristol larva. The populations in the less favorable media were qualitatively related to the generation times.

TABLE II

Typical generation times at 20°C for larvae of two strains of Caenorhabditis elegans

Culture Medium	Generation I time — days †	
	Bergerac strain	Bristol strain
A. GS-47 with 1% LPF-C ††	—*	7
B. GS-46 with 10% CEE	5	3
C. CEE + ALE 1 : 1	4	3
D. CEE + Oxoid liver 1 : 1 **	—*	6
E. ALE + LPF-C 1 : 1 **	—*	5
F. GM-22 with 10% [ALE + LPF-C 1 : 1]	—*	5

† Three individual replications; results with medium D confirmed in 3 repeated tests over 7 months.

†† Reported by NICHOLAS *et al.*, 1959, p. 233.

* Large adults developed in 3 to 5 days. These failed to lay eggs and showed vacuolated ovaries; a single larva was produced in one culture in medium F.

** Different levels of activity may be found in different preparations of the extracts.

DISCUSSION

The populations of each strain of *C. elegans* can be considered homogeneous, for, even though reproduction is sexual, the genetic composition of the offspring remains essentially constant since the species is almost entirely composed of self-fertilizing protandrous hermaphrodites. The occurrence of males is extremely rare in our cultures. The data are too few to permit any valid statistical evaluation of the frequency of appearance of males. Our results are, however, in accord with previous findings; MAUPAS (1900) reported 1.5 per 1000; NIGON (1949) 1.7 per 1000, and NIGON & DOUGHERTY (1949) 0.5 per 1000.

The tissue extracts used in these experiments were originally designed for maintance of axenic cultures of the free-living nematode, *Caenorhabditis briggsae*; the chemically defined media were designed and have been used to study the nutritional requirements of this worm. Unsupplemented, these chemically defined media do not support cultures of *C. briggsae*. Thus it was decided to determine whether either strain of the more rapidly growing species, *C. elegans*, could grow in these media without organic supplements. Larvae of the Bristol strain were found to grow better than those of either the Bergerac strain or *C. briggsae*; however, they did not reach maturity in unsupplemented media.

Both strains of *C. elegans* grew well in two of the media (B and C, Table II); but the Bergerac strain grew somewhat more slowly and yielded lower populations. Responses of the two strains were markedly different in two other media, D and E; the Bristol strain matured fairly rapidly and yielded large populations, while the individual larvae of the Bergerac strain grew to large adults, but did not reproduce. This difference is so clearly distinguishable that the possibility exists of demonstrating its genetic basis even though the complexity of the medium makes an understanding of the nutritional factors involved impossible at this time. It appears possible that this difference will be found to reside in the differential abilities of these two strains to utilize certain bound vitamins or co-factors from organic media.

The failure of isolated larvae of the Bergerac strain to reproduce in liver medium, E, is noteworthy since this medium is used for routine cultures of the axenic stocks. This suggests that quantitative differential nutritional requirements may be a function of temperature, as stock cultures are maintained at 17° C, experimental cultures at 20° C.

Additional evidence for the different cultural characteristics between the two strains is provided by the monoxenic cultures. Cultures of the Bergerac strain tend to die out when grown on nutrient agar in association with *Escherichia coli*. Cultures held for more than three weeks at 20°C produce, on transfer, only a few hermaphroditic-type adults; but these fail to produce offspring. This "non-fertility" has not been observed with the Bristol strain; successful transfers have been made from cultures after nine weeks. The less rich mineral agar (NIGON, 1949) permits slower development of the monoxenic cultures, and successful transfers of the Bergerac strain have been made at four weeks.

The difference between the nutritional requirements of these morphologically similar strains can perhaps be compared with the different growth requirements shown in culture by mutant strains of human carcinoma cells, which are also morphologically similar (PUCK, 1959). This may well be a case where differences in biochemical capabilities prove to be far more significant criteria for strain differentiation than morphological differences.

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ZUSAMMENFASSUNG

Über den Einfluss verschiedener Ernährung auf die Vermehrung zweier Stämme von Caenorhabditis elegans *in wirtsfremder Kultur*

Caenorhabditis elegans (Maupas) ist eine selbstbefruchtende protandrische zweigeschlechtliche Art. Zwei Varietäten von verschiedenen geographischen Gebieten, die als *Bergerac* und *Bristol* nach ihren Herkunftsgebieten benannt werden, wurden isoliert. Es wurden Unterschiede in ihren Nahrungsansprüchen gefunden, wie durch den Entwicklungsumfang ihrer Kulturen in verschiedenen Nährböden bewiesen werden konnte.

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SURVIVAL OF EMERGED LARVAE OF THE SUGAR-BEET NEMATODE (*HETERODERA SCHACHTII*) IN WATER AND IN SOIL

BY

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As reported by THORNE (1923), a small percentage of eggs containing second-stage larvae of the sugar-beet nematode (*Heterodera schachtii* Schmidt) may remain viable in the protective cysts for more than 6 years under field conditions in Utah. Because of the extensive survival of this nematode, profitable crops of sugar beets (*Beta vulgaris* L.) can usually be grown without obvious injury about 1 year in 5 on heavily infested fields if other susceptible crop plants are not grown on these fields during the rotation period.

Larvae of the sugar-beet nematode that have hatched from the egg and emerged from the cyst, however, have been reported to live for a much shorter time but there is no general agreement as to the length of time they may live. FILIPJEV & SCHUURMANS STEKHOVEN (1941), reviewing the literature to about 1940, cited reports giving limits of 40 and 140 days, but also pointed out that another author reported 50 % of the larvae alive after 379 days in sterilized soil in pots without plants. In a later review, FRANKLIN (1951) suggested that emerged larvae may live for "some months" and referred to a report that they may live for 2 years. The persistence of larvae is of practical interest since development of a method of causing the second-stage larvae (infective stage) to hatch and emerge from the cysts in the absence of host plants might make it possible to grow sugar beets more often in infested fields.

The experiments reported herein were designed to determine (1) the survival time of emerged, second-stage *H. schachtii* larvae in shallow dishes of plain water in the laboratory; and (2) how long the larvae can persist in soil outdoors without plants and still infect sugar beets.

MATERIALS AND METHODS

In obtaining larvae for the test of survival in water in the laboratory, about 100 fresh *H. schachtii* cysts were sieved from infested soil. These were placed in a Syracuse watch glass containing fresh root diffusate of sugar beet. After rapid larval emergence had begun, the cysts were transferred to another Syracuse watch glass also containing fresh beet-root diffusate and kept for 24 hours. Larvae for

the test were selected from this second watch glass and were, therefore, within 24 hours of the same emergence age. One hundred of these larvae were hand-picked and placed in each of twenty small Petri dishes (60 mm in diameter) containing about 15 ml of tap water each. Thus there were twenty replications of 100 larvae each, a total of 2,000 larvae in the test. The dishes with the larvae were stored in a dark, aerated cabinet in the laboratory at room temperature (about 24° C during the day and a little cooler at night). Every month fresh tap water was added to the dishes at which time the larvae were examined microscopically for general appearance and activity, and the active ones were counted. The test was continued until no larvae were active, even when stimulated with a fine needle.

For the test of survival of larvae in soil without plants, 15,000 fresh *H. schachtii* cysts were obtained as in the preceding test. About 750 of these cysts were placed in each of twenty Syracuse watch glasses containing 15 ml of beet-root diffusate. These watch glasses with the cysts were kept for 1 week, and then all emerged larvae were removed from the dishes and placed in water in a container. The volume was adjusted so that 25 ml contained 5,000 larvae, the number used to inoculate each crock.

Ninety, empty, 2-gallon, glazed crocks were sterilized and divided into fifteen series of six crocks each. All crocks were half-filled with sterilized soil moistened with water. Thirteen series (78 crocks) were sunk in the ground outdoors in a non-infested area near the laboratory at Salinas, California, in such way that the top of each crock was about an inch above the ground line. Sinking the crocks into the soil was done to minimize the possible effects of environmental changes. Twelve series (72 crocks) were inoculated by placing 5,000 larvae in each crock and immediately filling with well-moistened sterilized soil. The other six crocks were also filled with moistened sterilized soil, but were not inoculated. These six crocks (one series) were designated as Control III. The two series of crocks not sunken in the ground were handled as follows: six crocks, comprising Control I, were filled with sterilized soil, moistened with water, inoculated with 5,000 larvae per crock, planted with two young sugar beets per crock and then placed in the greenhouse. The six crocks of the remaining series (Control II) were treated in a similar manner except that none were inoculated. The soil in all crocks, whether with of without plants, was kept moistened with water during the test.

At monthly intervals for 1 year after initiation of the test, one series (six crocks) of the inoculated crocks was removed from the ground and placed in the greenhouse. Two young sugar beets were immediately planted in each crock and allowed to grow for 6 months. Then the beet tops were removed and the roots and soil of each crock were thoroughly mixed. An aliquot portion of soil from each crock was taken, washed and sieved twice to recover female nematodes and cysts which were counted and the number per crock was calculated. Control III was removed from the ground after 5 months and handled as the inoculated crocks above. The other two controls were processed in the same manner after the beets had grown in the crocks for 6 months in the greenhouse.

RESULTS AND DISCUSSION

During the first 2 months most of the larvae in water (Fig. 1) were active and apparently healthy. At the end of 3 months, however, only about half of the larvae

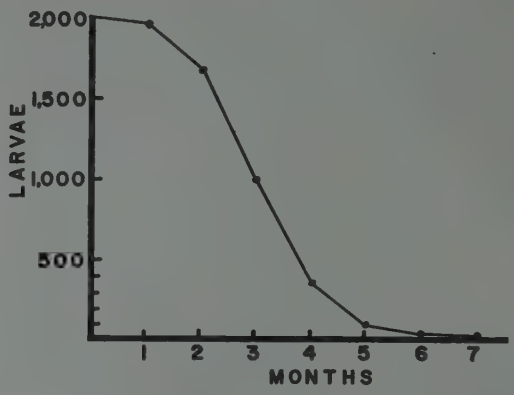

Fig. 1. Survival of emerged *H. schachtii* larvae in plain tap water.

TABLE I

Survival of emerged H. schachtii *larvae in soil-filled crocks without plants for varying periods of time, as indicated by number of cysts found in each of the same crocks later planted to sugar beets for 6 months*

Crock series [a]	Treatment of larvae: Months without plants	Subsequent months with sugar beet	Average number of cysts per crock
A	1	6	24,466
B	2	6	51,706
C	3	6	29,735
D	4	6	23,251
E	5	6	13,550
F	6	6	28,347
G	7	6	7,995
H	8	6	8,402
I	9	6	3,730
J	10	6	2,492
K	11	6	0
L	12	6	1,028
Control I [b]	0	6	40,235
Control II [c]	0	6	0
Control III [d]	5	6	0

[a] Each series composed of six crocks. Each crock initially inoculated with 5,000 larvae.
[b] Inoculated with 5,000 larvae per crock and beets planted at initiation of test.
[c] Not inoculated; beets planted at initiation of test.
[d] Not inoculated; kept 5 months with other crocks before being planted to sugar beets.

were active, and some of these were transparent, indicating that the food reserves in their intestinal cells were depleted. The decrease in the number of active larvae continued so that at the end of 6 months only 29 of the original 2,000 were still visibly active. At the end of 7 months all larvae were apparently dead.

Survival of emerged larvae in soil without plants (Table I) is considerably longer than in water. However, a sharp drop was noted in the number of cysts per crock at the end of 7 months (crock series G) compared with the preceding months. By the end of 11 months (crock series K) no cysts were found, indicating that larvae in sufficient numbers to establish an infection did not survive despite the fact that a total of 30,000 larvae were used initially to inoculate the six crocks of the series. As females of *H. schachtii* apparently do not reproduce parthenogenetically (GOLDEN, 1959), at least two larvae per crock and probably many more must survive and develop in order to establish an infection. Cysts were found, although in relatively small numbers, in two crocks of series L after the larvae were in the soil without plants for 12 months. Thus, at least a few of the original 30,000 larvae in the crocks of series L remained alive and infective for 12 months, although not enough of those in series K remained alive to establish an infection after only 11 months.

Although not entirely conclusive, the results seem to indicate that the larvae would probably not survive and remain infective appreciably longer than 12 months under the conditions of this experiment. Only a very small percentage of the nematodes survived for this period and, furthermore, most of the larvae apparently were not infective after the seventh month.

One reason for larvae of the sugar-beet nematode becoming non-infective is exhaustion of their food reserves as noted in the larvae kept in water. The length of time this reserve lasts is greatly influenced by activity of the larvae, which in turn depends on soil temperature and moisture. Thus the larvae might live longer in a cooler climate than in that of Salinas, California, or even less in soil drier than that used in this experiment. The effects of different environmental conditions might account for the difference between the results of this experiment and those cited in the introductory section.

The survival of *H. schachtii* larvae in these experiments agrees with the results of GOLDEN (1956), who found that some adult specimens of a spiral nematode (*Helicotylenchus buxophilus* (Golden) Perry) were able to persist in potted soil without plants as long as 8 months but not for 12 months. The persistence of the sugar-beet and this spiral nematode is considerably longer than has been reported for certain other nematodes. BIRCHFIELD (1957) reported that the burrowing nematode (*Radopholus similis* (Cobb) Thorne) survived only 66 days in water and agar and was not recovered from infested soil and roots after 4 months. More recently, VAN GUNDY (1959) found that second-stage larvae of *Hemicycliophora arenaria* Raski died within 1 week in water, that specimens in intermediate stages remained active 2-3 weeks, and adult males did not live more than 2 weeks while adult females remained alive and active for 4-5 weeks without feeding.

From a practical standpoint, the results of these experiments indicate that if larvae in cysts of the sugar-beet nematode in field soil could be stimulated to emerge in the absence of host plants, a very large percentage of those emerging would be non-infective within a year.

ZUSAMMENFASSUNG

Überleben geschlüpfter Larven des Rübennematoden (Heterodera schachtii) *in Wasser und Boden*

Aus 2000 geschlüpften Larven II des Rübennematoden, die in Leitungswasser im Laboratorium gehalten wurden, waren am Ende von 6 Monaten noch 29 am Leben; keine Larve überlebte den siebenten Monat. Einige wenige der 5000 geschlüpften *Heterodera-schachtii*-Larven, die zur Infektion von mit Erde gefüllten Töpfen ohne Pflanzen benutzt wurden, blieben lebendig und in zwei Töpfen von 6 in einer Reihe für 12 Monate infektionsfähig. In einer anderen ähnlichen Topfreihe, die auch am Anfang mit 5000 Larven infiziert war, wurde aber nach 11 Monaten keine Zyste gefunden. Die Ergebnisse deuten an, daß die geschlüpften Larven unter den Bedingungen des Experiments wahrscheinlich nicht viel länger als ein Jahr am Leben und infektiös geblieben sind.

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STUDIES ON THE NEMATODE SOIL FAUNA OF SUGARCANE FIELDS IN MAURITIUS
5. NOTES UPON A PARASITE OF ROOT-KNOT NEMATODES [1])

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During examination of *Meloidogyne javanica* (Treub) and *M. incognita acrita* Chitwood, obtained from sugarcane roots in Mauritius, the bodies of several females were seen to be filled with the spores of a micro-organism. Subsequent study of this organism led to the conclusion that it is parasitic and also, judging by the frequency of infected nematodes, that it may have an important role in the natural regulation of *Meloidogyne* populations.

The organism could not be identified but is it believed to be a Sporozoan belonging to either the Haplosporidia or Microsporidia. The spores seem to resemble those of *Duboscqia penetrans* Thorne, a parasite of *Pratylenchus brachyurus* (Godfrey), but certain forms which occur during development, as described below, are quite unlike those of any micro-organism recorded previously from nematodes. The observations made are by no means exhaustive but in view of the economic importance of root-knot nematodes some account of them seems desirable and they may serve to draw attention to the parasite elsewhere, for it is unlikely to be of restricted distribution.

EFFECTS OF THE PARASITE UPON THE HOST

Only adult females of *Meloidogyne* were examined and in these parasitism results eventually in the development of innumerable tightly-packed spores which completely fill the body. An infected host in this extreme condition is distinguished easily from healthy individuals because the spores impart to the body a milky white colour which is distinctive and evident under the low power magnification of a stereoscopic microscope. Healthy hosts are by contrast of a dull colour and more or less translucent. The spores are released and flow from the host body as a milky liquid if the cuticle is pricked or ruptured by pressure. The density of the spores is suggestive of their being held under pressure by the host cuticle but the size and shape of infected and healthy individuals are similar and neither distention nor distortion of the former is evident. The external appearance of infected

[1]) Previous studies appeared in *Occ. Paper Sug. Ind. Res. Inst. Mauritius*, Nos. 1-4, 1958-60.

females which contain few or no spores is identical with that of healthy females. The internal effect of parasitism appears to be the complete destruction of all host organs and tissues, only the valvular bulb of the oesophagus and its connection with the stylet remain as distinct structures.

MORPHOLOGY AND DEVELOPMENT OF THE PARASITE

Female *Meloidogyne* were removed intact from roots of sugarcane and mounted directly in the modified Berlese Fluid of REED, HUTCHINSON & RACE (1957). Pressure was applied to the coverslips to burst the bodies of the nematode and expel their contents into the mounting medium (Fig. 1, a). The preparations were then examined with a phase contrast microscope which was essential owing to the absence of staining and the clearing action of the mounting medium. This simple and rapid technique was adopted originally to examine female perineal patterns of *Meloidogyne* for purposes of identification and it was during the course of that work the micro-organism was detected. The technique fortuitously proved excellent for viewing the various stages of the parasite with high power oil immersion objectives and no other method of preparing material for study was attempted.

It proved impossible to determine the correct sequence of events during development of the parasite from the appearance of the various forms which may be distinguished. There are, however, clear affinities between many of the forms which enable them to be grouped as representatives of a particular phase of the life cycle. Two such groupings, each comprising several stages and each representing a separate phase of development, were possible. The spore and the forms comprising these two groups will be described separately as phase I, II and III, respectively.

Phase I. The spore (Figs 1, b; 2, a, b; 3, a, b) is the most conspicuous stage of the life cycle and is of distinctive form and structure. It measures 5 μ by 4 μ and appears round from above or below but in profile the upper half is seen to be dome-shaped and the lower half convex-conoid. There is a large, thickened, spheroid structure at the top of the spore which will be termed the polar capsule but its nature and function are obscure. This capsule gives the spore, when viewed from above or below, the appearance of possessing a large, dense, central nucleus. The wall of the spore around the polar capsule is strongly thickened so that the top half of the spore is, in effect, a thickened cap. Apart from the polar capsule, the spore has no evident internal structures, its contents being clear and transparent. There is no trace of a polar filament.

The thickened cap of the spore including the polar capsule was not infrequently seen as a separate entity (Fig. 3, c), as if detached. This may be indicative of the way in which the spore releases its contents.

Phase II. A number of forms, all basically similar, are included here (Figs 2, c, d; 3, d-k). Each consists of ovoid or elongate-ovoid bodies connected together by a central structure. The number of ovoid bodies, or buds, borne by the central

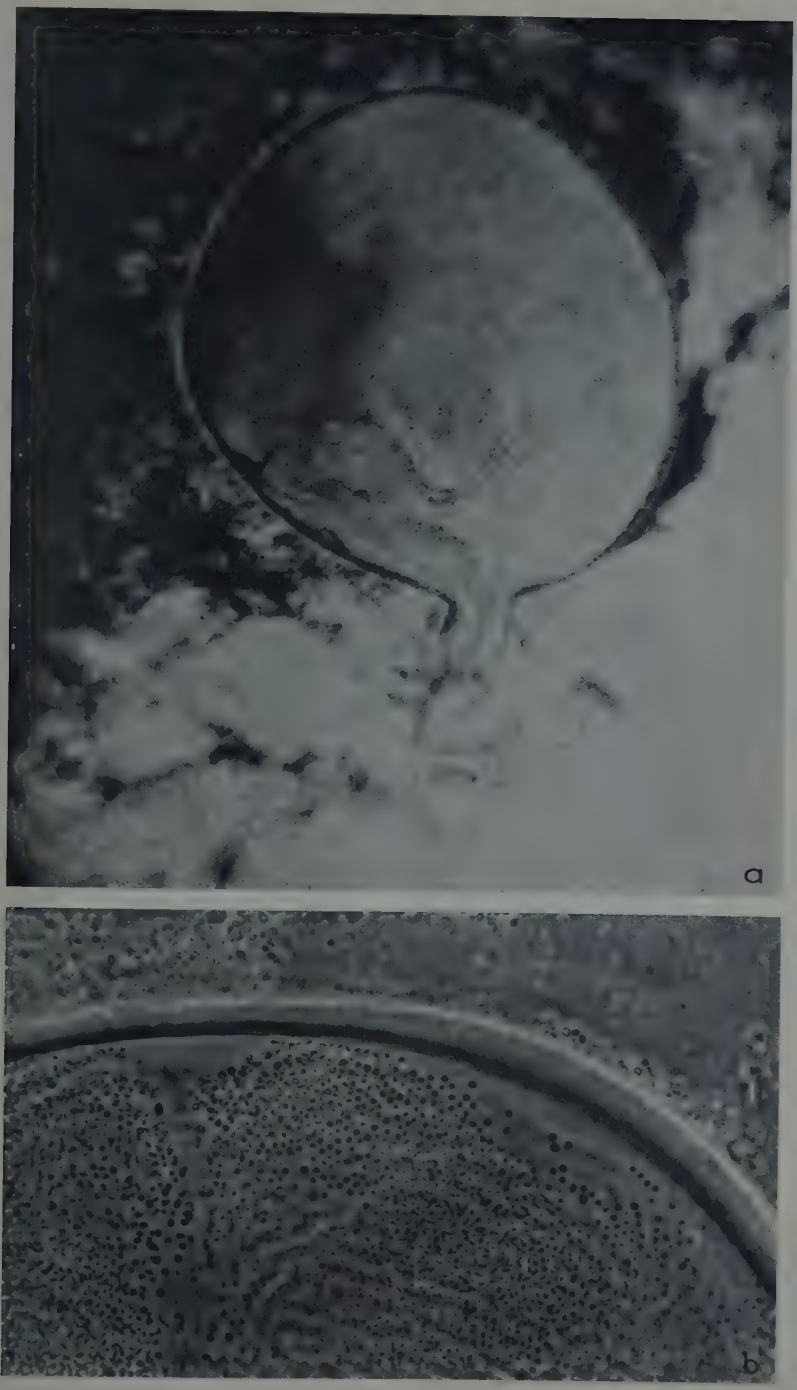

Fig. 1. (a) adult female *Meloidogyne* with cuticle ruptured by pressure. The spores which filled the body have been partially expelled into the mounting medium and appear as a white mass. Dark ground illumination. (b) the same viewed under higher magnification and phase contrast. The thick band is the cuticle of the nematode.

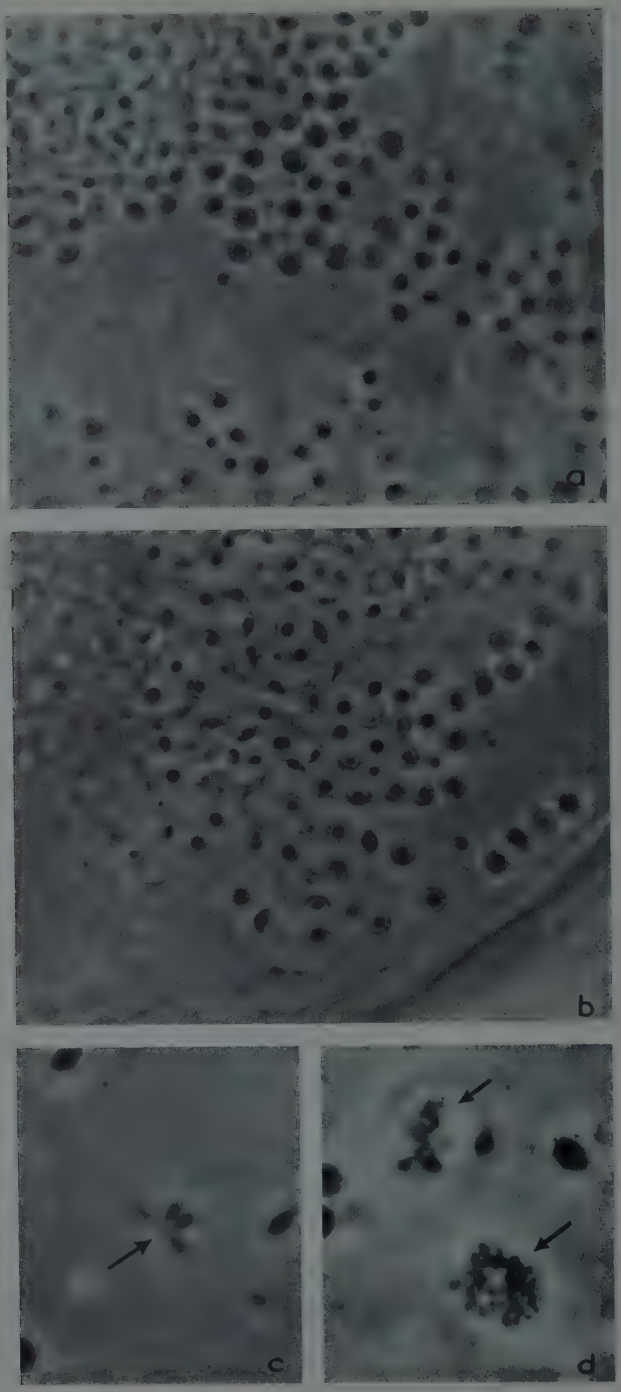

Fig. 2. (a) and (b) spores from body of adult female *Meloidogyne*. Phase contrast. (c) and (d) developmental stages of the same organism. Phase contrast.

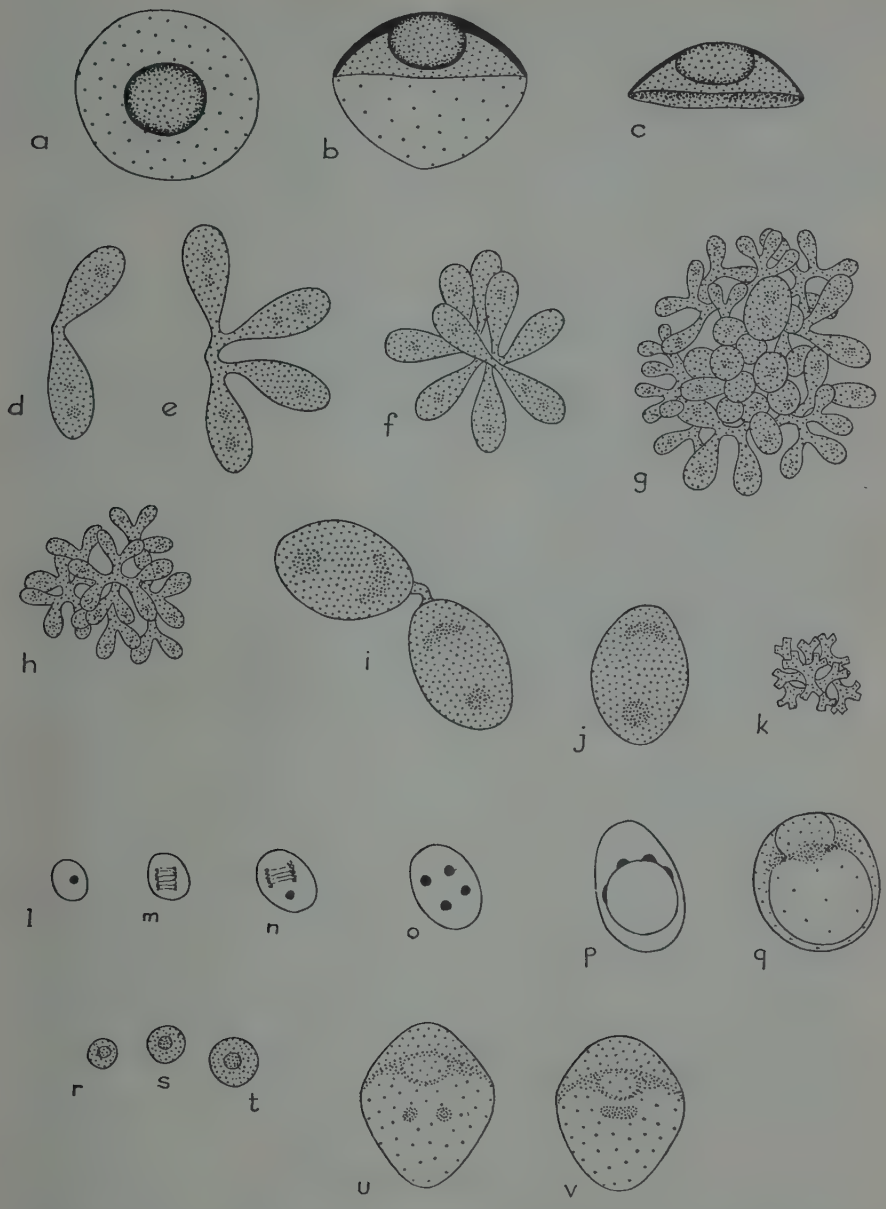

Fig. 3. Stages of a sporozoan parasite from adult female *Meloidogyne* (a) spore, seen from above or below. (b) same, in profile. (c) detached cap of spore. (d)-(k) forms comprising phase II of development. (k) connecting structure after detachment of the buds. (l)-(p) forms comprising phase III of development. (q)-(v) other forms observed.

structure varies considerably, as also does the size of the buds. There may be two or several large buds, many small buds, or many small and large buds connected together. The central connecting structure is dichotomously branched when the number of buds exceeds two. The overall size of a form with a large number of of buds is twice or thrice the size of a spore and it appears as an ill-defined but discrete and often more or less spherical mass of linked bodies (Figs. 2, d; 3, g). The cytoplasm of both buds and connecting structure is dense and opaque but one or two darker nuclear (?) bodies are usually evident in the buds.

These forms represent a phase of the life cycle when multiplication is rapid and appears to occur by the successive division of each ovoid body (bud) into two daughter bodies which remain connected at their bases. These buds eventually break away and the central connecting structure is left as a residual body (Fig. 3, k). It was not possible to determine the exact steps of this process. It is uncertain whether the buds increase in size before breaking away, that is, whether the end product is small or large detached buds. It is also possible that the larger groups split up and that some of the forms seen were fragments freed by breakage of a link in the connecting structure. It is, however, quite clear that multiplication occurs by the production of buds upon a dichotomously branched body and that the large number of buds so produced become detached as daughter forms.

Phase III. The forms comprising this stage are ovoid cells with well defined nuclei and clear transparent cytoplasm (Fig. 3, l-p). They are of various size and contain one to four nuclei. Nuclear division is often distinct. The larger cells frequently contain a clear spherical structure and the nuclei are arranged around its periphery. The cells are sometimes aggregated in small groups but most are solitary and there is little to indicate cell division, as opposed to nuclear division. Other forms which have been observed are illustrated (Fig. 3, q-v).

The relative abundance in infected hosts of the stages which comprise phases I, II, and III varies. Hosts which are full of spores contain very few forms of phase II but often contain many forms of phase III. When spores are rather less abundant the forms of phase II are usually plentiful but they were found to be most numerous in some hosts where spores seemed to be absent. In slightly (recently?) infected hosts only phase II forms were seen, that is, when the parasite was seen in very small numbers in a host it was present only as forms of phase II, as far as could be determined. These observations coupled with the morphological characteristics of the various forms point to the conclusion that the life cycle is initiated by the detachment of the spore cap to release a form which multiplies prolifically by budding (phase II) and the daughter forms then develop by complicated nuclear rearrangement (phase III) into spores.

MODE OF TRANSMISSION

The spore is the most robust stage of the parasite and seems the only stage which might be expected to survive conditions outside the host. It is also the end product of parasitism in the sense that the bodies of infected hosts ultimately

contain little else than a mass of spores. Thus, although direct evidence is lacking, it may be inferred that dispersion of the parasite is effected by the spores following their release by rupture or dissolution of the host cuticle. The spores have no apparent power of locomotion but this is compatible with the prodigious number which develop in each infected host. The spores are undoubtedly carried passively in water and soil movements.

The manner in which the parasite enters a host is conjectural but it is unlikely that the spore enters intact.

INCIDENCE OF PARASITISM

A total of 174 female *Meloidogyne* were removed from roots of sugarcane growing in the field where infected individuals were first obtained. The soil was sandy and the cane was badly attacked by the nematodes. Microscopic examination showed that 34 % of the nematodes were infected and, since slightly infected hosts may have been overlooked, this figure is a conservative estimate of the true incidence of parasitism.

DISCUSSION

Dolfuss (1946) states (pp. 66,67) that many Protozoa living in nematodes seem to be harmless and that none should be considered parasites without good reason. He also states (p. 79) that the identification of Protozoan parasites of nematodes is very often impossible owing to the inadequate knowledge of such forms which exists at present and that many which have been determined as Sporozoa undoubtedly belong to other groups ("Il y a des erreurs probables et d'autres manifestes").

While there seems no reason to doubt the parasitic nature of the micro-organism which has been described, for its multiplication in *Meloidogyne* females progresses until the nematodes become literally mere bags of spores, its identity is uncertain. The spores and the mode of development, as far as this is known, suggest a Sporozoan belonging to either the Microsporidia or Haplosporidia while the apparent absence of a polar filament and the way in which the spore seems to open are characters which indicate the latter of the two groups. The forms of development which have been referred to collectively as phases II and III possibly represent schizogony and sporogony, respectively. However, the structure of individuals of phase II do not seem to resemble the schizonts of any known Sporozoa.

Thorne (1940) described a parasite of *Pratylenchus brachyurus* and named it *Duboscqia penetrans*, although its identity is questionable. The spores which he figured seem to have a resemblance to those of the organism under discussion but the similarity of the two forms does not extend further if Thorne's provisional account of the life cycle of *D. penetrans* is correct. It should be remarked, however, that Thorne's data upon the developmental stages of the parasite he studied seem very incomplete and his figures are inadequate. Kuiper (1958) reports from

Holland the presence of Protozoa similar to *D. penetrans* in several species of Tylenchoidea, including *Meloidogyne arenaria,* and this is apparently the only previous record of Sporozoa in *Meloidogyne* species.

The author is indebted to Dr. J. M. Watson, Director of the Commercial Bureau of Helminthology, for kindly providing literature which was required and to Mr. F. G. W. Jones and Dr. J. B. Goodey of Rothamsted Experimental Station for carefully reading the manuscript.

RÉSUMÉ

Notes concernant un parasite des Nématodes des racines

L'auteur décrit un microörganisme parasitaire, observé dans des femelles adultes de *Meloidogyne javanica* et *M. incognita acrita* provenant de racines de canne à sucre de l'Ile Maurice.

Cet organisme ne put être identifié avec certitude et paraît appartenir à l'ordre des Microsporidia ou Haplosporidia. Les spores ressemblent en général à ceux de *Duboscqia penetrans* Thorne, mais certains stades évolutifs (probablement des schizontes), ne ressemblent à aucune des formes connues de sporozoaires parasitant les nématodes. Le corps de l'hôte infecté peut être rempli de spores, les tissus internes étant apparemment complètement détruits.

L'incidence du parasitisme des femelles pouvant être élevé (34%), cet organisme parasitaire pourrait être un ennemi naturel important des nématodes des racines.

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STUDIES ON THE BIOLOGY OF *RADOPHOLUS SIMILIS* (COBB, 1893) THORNE, 1949

PART III

Embryology and Post-Embryonic Development

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EMBRYOLOGY

When burrowing-nema infected plants were lifted from the soil, their roots washed clean and immediately dissected, eggs in all stages of development were generally recovered. They were slightly kidney shaped and varied in size from 58-70 μ long by 23-28 μ wide. The two poles were equally rounded (Fig. 1, B). The outside cover consisted of a tough eggshell, surrounding closely packed, round globules of uniform diameter. Where the egg protoplasm had shrunk away from the walls, presence of a vitelline membrane was revealed, which left the so-called perivitellar space between the vitelline membrane and the embryo. This shrinkage also allowed the vitelline membrane to separate from the egg-shell, giving rise to an extra-vitellar cavity (Fig. 1, C).

When embryonated eggs were placed in an aqueous solution of five percent gentian violet, the vitelline membrane was not penetrated by the stain and the blastomeres remained uncolored. After slightly heating the egg or after treating it with ordinary fat solvents like acetone, the gentian violet colored the entire egg dark violet, indicating the possible presence of lipoidal substances in the vitelline membrane.

The undivided egg was the most common stage of development found upon dissection of infected roots. As much as 30 percent of all eggs recovered could be in this stage; but, the greater part did not develop normally, particularly the slightly longer and narrower (length/width exceeding 2.5), more strongly bent and darker colored eggs failed to cleave (Fig. 1, A).

After cleavage had started, the presence of polar bodies in burrowing nema eggs became apparent. Usually the first polar body was observed outside the vitelline membrane in the extra-vitellar cavity. Occasionally this polar body divided so that two such bodies were observed in the extra-vitellar cavity. Since these polar bodies occurred outside the vitelline membrane it follows that they were formed before the vitelline membrane had been laid down. The second polar body, only rarely observed, occurred always singly inside the vitelline membrane and was

therefore the last to be formed. Although many eggs were observed where the polar bodies had disappeared in the early stages of development, sometimes both, or more often one of the extra-vitellar polar bodies remained present up to the time of hatching.

Figure 1. Eggs of *R. similis* in various stages of development.

In the early "tadpole" stage of development (Fig. 1, M) the embryo could not yet reverse itself completely. At this time the polar bodies were invariably located at the pole corresponding to the anterior end of the embryo.

The undivided egg (P_0) indicated the beginning of its first cleavage by a continuous movement and rearrangement of its protoplasm. The first division took place perpendicular to the long axis of the egg, resulting in a large posterior

blastomere (P_1) and a slightly smaller anterior cell (S_1). The polar body was always located at the anterior pole. Eggs of *R. similis* differ in this respect from those of *Heterodera schachtii* Schmidt. In *Heterodera schachtii* the polar body is located at about the middle of the egg, opposite the first plane of division, according to STRUBELL (1888). In the two-celled stage, the two nuclei showed up as large, rounded, clear spaces, one approximately in the center of each cell.

During the second cleavage the anterior cell (S_1) divided into an anterior cell (A) and a center cell (B), the plane of division usually being parallel to the first plane, perpendicular to the long axis of the egg. The two new cells were of about equal size and about half as large as (P_1). Each cell contained a large nucleus. The cells were arranged in tandem but could shift their position, leaning to the ventral or dorsal side, without, however, disturbing their respective order. The next division in this cleavage divided the posterior cell (P_1) into two blastomeres, (P_2) and (S_2), bringing the total number of cells to four. In cases where the plane of division was parallel to the existing ones a tandem arrangement was mostly the result, in which (P_2) was the cell at the posterior pole (S_2) its neighbour (Fig. 1, E). However, considerable shifting could take place. The exterior cells (A) and (P_2) changed their respective location only slightly, but (S_2) often would move to a more central, ventral position while (B) took up the central, dorsal space. More frequently, however, variation from the tandem arrangement was due to a plane of division not parallel to the existing ones but at some angle. This allowed many configurations to exist one beside the other, without one being the result of another (Fig. 1, E, F, G).

Development immediately following the four-celled stage was characterized by several rapid divisions at the anterior end of the embryo, resulting in smaller cells at this pole as compared with those at the posterior end. The total number of cells as shown in Figure 1, J was eight: one large posterior cell, two large central cells, three smaller anterior cells, and two small lateral cells, one on each side of the embryo.

In the subsequent cleavages the picture became more obscure. Not only did the total number of cells increase rapidly, but also several cells divided simultaneously with the cells of each given group having their own cleavage "rhythm", different from those of other cell families. Figure 1, K shows a fourteen-cell stage and Figure 1, L shows a forty-one cell stage.

The early "tadpole stage" is shown in Figure 1, M. The ectoderm is being differentiated by small angular cells surrounding an inner mass of larger cells. A definite difference in density of globules identified a hyaline anterior end as compared with a dark colored posterior zone.

In subsequent stages elongation of the embryo took place, resulting in one or more flexures. At this stage the embryo was capable of turning inside the egg membranes. It is generally accepted that the first molt concludes the first larval stage. Exactly where the embryonic development ended and the first larval stage began was impossible to determine. As is shown later (p. 49) the body length

of molting, first-stage larvae was the same as the length of fully developed second-stage larvae. It seems, therefore, not unreasonable to assume, that the first molt takes place when the elongation process of the embryo had been completed.

Figure 1, O shows a first-stage larva molting. Occasionally a fully formed stylet was present in the molting animal with molted cheilorhabdions (stylet guide) and prorhabdions (conoid or insertable part of the stylet) inside the loose, molted cuticle at the head end. Generally, however, the molt took place before a complete stylet had been formed. Prorhabdions were often present in the animal itself at this time which might indicate that the prorhabdions are formed prior to the meso-metarhabdions (stylet shaft) and telorhabdions (stylet knobs). The cephalic region, although indicated by a slightly different light-refraction, was not sclerotized [1]) at the time of molting. The median bulb was sometimes differentiated, with the valve in an asymetric position, anterior to the middle of the bulb. Generally all four cells were clearly present in the genital primordium at this stage.

Further development resulted in a fully-formed second-stage larva inside the egg shell, characterized by a well-defined, sclerotized cephalic region, a strong stylet, and a fully differentiated esophageal region.

The time necessary for embryonic development in spring water varied from a little over four days to eleven days depending on the temperature. The use of tapwater proved detrimental to the development of burrowing nema eggs, but natural spring water obtained from Blue Springs, Florida, did not harmfully affect the nemic embryology.

POST-EMBRYONIC DEVELOPMENT

As has been shown by CONTE (1900), CLAPHAM (1930), BINGEFORS (1957), VAN WEERDT (1958), measurements of actual sizes of nemas, expressed in absolute terms such as microns, are not necessarily reliable indications of the stage of development, because size is influenced by environmental factors. Studies were therefore made of the differentiation and development of the gonads to try to use the resultant information as a basis for determining the various larval and adult stages of *R. similis*. A possible correlation between the stage of development of the gonads and the size changes of those rigid parts of the nema which are generally replaced at each molt, such as the sclerotized cephalic region and the stylet, was also investigated.

MOLTING

When fully embryonated larvae inside the egg shell were placed in water on a microscope slide and covered by a cover slip, they could be hatched artificially by applying slight pressure on the cover slip with the aid of a needle, which broke the egg membranes. The intestines of these young second-stage larvae were almost completely devoid of globular material, which might explain why such freshly hatched

[1]) The term sclerotized is used to indicate hardening without signifying the chemical composition.

larvae succumbed rapidly in water. When infected roots were dissected the second-stage larvae recovered looked wellfed with a reserve storage of granular materials. It was therefore assumed that in their natural habitat, plant roots, the hatched larvae fed and stored excess food material before going into their second molt.

Larvae ready to molt initiated a transition period by becoming sluggish in their movements. Later they became quiescent with occasional contractions and finally straight and immobile. The basal part of the stylet (shaft and knobs) disappeared, and the esophagus, including the median bulb and the valve, became disorganized. Subsequently the animals contracted. The anterior extremity of the nemas pulled back from the cephalic frame work, at first a little on each side of the conoid part of the stylet, later also at the base of the prorhabdions. In most cases the stylet-tip remained surrounded by the cephalic framework, but occasionally the conoid part of the stylet was present, free inside the loose cuticle. In addition to continued contraction of the nemic body, which was indicated by progressively stronger striations, with the annules closely together, the old cuticle apparently stretched, since its annulation became more and more indistinct and in some cases disappeared completely. These combined phenomena caused the cuticle to loosen increasingly around the body. In the final stages of the transition period the new organs became clearly defined, the new conoid part of the stylet, the cephalic framework and the lining of the terminal excretory duct became sclerotized. Molted parts included the old cephalic framework, the cheilorhabdions, the prorhabdions, the lining of the two amphidial tubes (Fig. 2, A, arrow), the lining of the terminal excretory duct (Fig. 2, B, arrow), and the lining of the rectum (Fig. 2, C, arrow), all of which, with the exception of an occasional loose conoid part of the stylet, remained attached to the molted cuticle. The cast cuticle broke somewhere in the esophageal region in most observed cases. Occasionally the break in the cuticle was not complete and the nema had to escape sideways.

The recovery of specimens showing superimposed molts by dissection of infected roots was very rare. However, most of the molting specimens recovered by incubating infected roots for several days, as described by YOUNG (1954), showed one or more superimposed molts. Figure 3 shows three superimposed molts in the case of a male burrowing nema kept in water for several days. Complete shedding of the old cuticle was therefore not a prerequisite for further development.

These observations seem to be in agreement with the results obtained by others. SCHWARTZ (1924), investigating the life history of the cattle hookworm, *Bunostomum phlebotomum,* found that third-stage larvae obtained from solid culture media had retained only the second cuticle ("protective sheath"), the first cuticle having been discarded. Third-stage larvae obtained from liquid cultures showed two sheaths, indicating that the first as well as the second sheath had been retained. LINFORD & OLIVEIRA (1940) studied molting *Rotylenchulus reniformis* specimens placed in water or liquid media and found that the nemas "developed through a series of at least three superimposed molts except that, lacking a firm substratum, they remained encased within their multiple last cuticles."

DEVELOPMENTAL STAGES

The first stage larvae were studied by selecting individuals which were judged to have completed their elongation, including specimens undergoing their first molt. Since these larvae were still enclosed within the egg membranes, the incubation method of recovery as described by YOUNG (1954) could not be used. Infected roots were therefore dissected in water and the selected eggs transferred in water to a microscope slide with the aid of a capillary glass tube. A coverslip

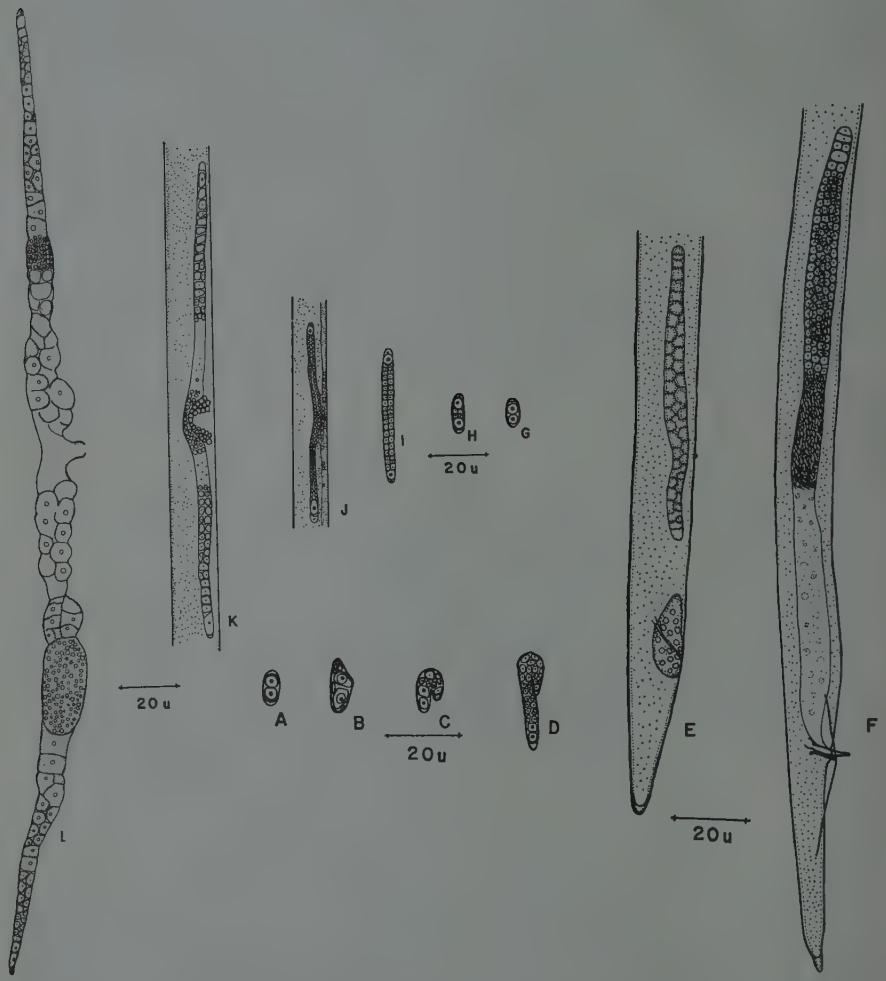

Figure 4. Stages in the development of the male (A through F) and female (G through L) gonads. A: first molt; B: second molt; C and D: third molt; E: fourth molt; F: adult males; G: first molt; H: second molt; I and J: third molt; K: fourth molt; L: adult female.

was then placed over the eggs. Artificial, premature hatching was achieved by applying a slight pressure on the coverslip which ruptured the egg membranes. The coverslip was then removed and replaced, supported by threads of fine glass wool.

Larvae of post-hatching age were obtained by dissection of infected roots or by incubating the roots as described by YOUNG (1954). The development of the genital primordia was traced, using the following staining method. Larvae were placed in a five percent solution of acid fuchsin in water and heated for eight minutes at 50° C. They were then stored overnight in the staining solution at room temperature. Over-staining could be corrected by addition of weak alkali. The nemas were mounted in two percent formalin. Glass wool was used to support the coverslip. By this procedure the nemas were stained pink except the genital primordia and the cloacal primordia of the males, which were stained red.

After a comparative study had been made involving hundreds of molting larvae, four molts could be accounted for, one occurring before hatching, and three after the larvae had hatched from the eggs.

First-stage larva. — Larvae, hatched artificially, ceased all movement after a few weak contractions and succumbed rapidly, usually within six hours. They were rather wide for their length, measuring from 225-270 μ long and from 13-15 μ wide, with the greatest width occurring about one-third from the anterior end.

The cephalic framework, although somewhat differentiated, was not yet sclerotized at this stage (Fig. 1, O). It measured 2-2.5 μ long and 5 μ wide. A complete, refractive stylet was occasionally present in the molting specimens, 11-12 μ long. Generally, however, only the conoid part of the stylet was observed.

The excretory pore was situated from 40-55 μ from the anterior end, immediately behind the hemizonid. In most cases the esophageal region was not yet differentiated although sometimes a median bulb could be distinguished in the older individuals.

The genital primordium consisted of four cells. Two large, rounded, centrally located, germinal cells, each with a conspicuous nucleus, were enclosed by two smaller epithelial cells as shown in Figure 4, A and 4, G. The genital primordia measured 9-10μ long and 4-5 μ wide and were located about 100 μ from the tail tip. Male and female larvae could not be distinguished at this time. CHITWOOD & CHITWOOD (1950) stated: "the genital primordium is identical in that it consists of two germ cells and two epithelial cells in the first stage larva of both sexes of all nemas studied." *Radopholus similis* is apparently no exception.

Second-stage larva. — Since this stage comprised the development between the first and the second molt, the early development of these second-stage larvae occurred while they were still enclosed within the egg shell.

These second-stage larvae of *prehatching* age did not differ appreciably as to length and width from the fully elongated first-stage larvae. The cephalic framework was sclerotized, however, measuring 2-2.5 μ long and 5-6 μ wide. A com-

plete stylet was also present measuring 11-12 μ long. The esophageal region, including the median bulb, was clearly differentiated. The genital primordia were the same as described for the first stage larvae.

The second-stage larvae of post-hatching age were generally longer but narrower than before hatching. They measured 244-347 μ long and 12-14 μ wide. The intestine, fairly translucent at the time of hatching, became more and more opaque and filled with granules. The cephalic framework did not change in size during this larval stage and measured for most animals 2.5 μ long by 6 μ wide. The stylet lengths observed varied from 12 μ to 14 μ, which were, on the average, slightly larger than those of the second-stage larvae before hatching.

In the later part of this stage and during the second molt the genital primordia showed their first development, which proceeded in two different ways. In the case of female larvae the two germ cells became separated by from two to six somatic cells, one germ cell entering each gonad (Fig. 4, H). The female genital primordia ranged from 9 μ to 22 μ in length.

In the case of the male larvae the germ cells remained together, but from two to four somatic cells separated the germ cells from the *anterior* epithelial cell (Fig. 4, B). The male genital primordia started their development therefore by growing out towards the anterior end of the nema; they ranged from 9 μ to 16 μ in length. Although the positions of the genital primordia varied, in both males and females, from about 120 μ to 140 μ from the posterior end, the differences in development made it possible to distinguish the sexes. These differences in development of the gonads remained the only reliable way to distinguish males from females prior to the fourth molt.

Third-stage larva. — Third-stage larvae ranged in length from 360 μ to 550 μ and in width from 14 μ to 18 μ. The cephalic framework had been replaced during the second molt by a larger one measuring 2.5-3.5 μ long and 6.0-7.5 μ wide, usually 3 × 7 μ. The stylet measured 15-16 μ. The base of the median bulb was located about 50-60 μ from the anterior end. The tails measured 45-60 μ long.

In females the two germinal cells of the genital primordia were pushed farther apart by the formation of additional somatic cells between them. In this manner the gonad elongated both anteriorly and posteriorly, with its center remaining in about the same position. The width of the gonads during this elongation process remained fairly constant, while the length observed increased from 13 μ to 80 μ. Two of these stages are shown in Figure 4, I and J. The individual somatic cells became smaller in size as their number increased. In the later stages of the third molt, several cells were differentiated in the ventral chord opposite to the middle of the gonad. Their shapes and staining properties were very similar to those of the somatic cells of the gonads, with which they intermingled at the border line. These cells arising from the ventral chord eventually gave rise to the vagina in the mature animal. The female genital apparatus is therefore of double origin, mesodermal and ectodermal.

2

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Figure 2. Molting male *R. similis*. Structures cast include A: cuticle, cephalic framework, cheilorhabdions, prorhabdions, lining of the amphidial tubes (arrow); B: lining of the terminal excretory duct (arrow); C: lining of the rectum (arrow) (1500 X).

Figure 3. Three superimposed molts in a male burrowing nema kept in water for several days.

Further development of the male gonads showed considerable variation. However, in all cases the anterior part of the genital primordium continued to form additional somatic cells. The outgrowth, which initially was directed anteriorly and took place mainly on the dorsal side of the genital primordium, now continued towards the ventral side of the animal and proceeded in a posterior direction (Fig. 4, B; 4, C; and 4, D). Soon afterwards the genital primordium straightened out from its bent position and continued elongation towards the posterior end (Fig. 4, E). Male gonads of the third larval stage ranged from 14 μ in the early stages to 92 μ at the completion of the molt.

The double origin of the male genital apparatus also became apparent in this third larval stage through the formation of the cloacal primordia developing from rectal, epithelial cells. The position of the cloacal primordium remained restricted to the rectal area. Mostly it was triangular in shape measuring about 27 μ long and about 12 μ wide (Fig. 4, E).

Fourth-stage larva. — The fourth-stage larvae ranged in size from 445-595 μ long and from 16-24 μ wide. The cephalic framework varied from 3.5-4.5 μ long and from 7-9 μ wide, but usually measured 4 μ long and 8 μ wide. The stylet was usually 17 μ long, although a range from 16-18 μ was observed.

The gonads of the females in this stage became definitely attached to the body wall as a result of a proliferation of the cells of the body wall, which connected with those of the genital primordia. In between these proliferating cells, which stained a deep red, a clear hyaline spot was observed, indicating the position of the vaginal primordium. In the gonads the region of the uteri and ovaries became clearly differentiated during this stage. The anterior parts of the gonads ranged from 84-115 μ in length, the posterior parts measured from 90-140 μ.

The development of the male gonad in the third and fourth larval stage is subject to considerable variation. The gonads ranged from 196-245 μ in length. In this stage the connection between the gonad and the cloacal primordium was established by the growth of the former in a posterior direction until it reached the cloacal primordium. This latter primordium increased only slightly over the 27 μ by 12 μ reported for the third larval stage. The cloacal primordium gave rise to the spicules and gubernaculum during this stage. The caudal alae were also formed during this time.

With the exception of the genital apparatus, which for the fourth-stage larva was essentially the same as that described for the molting third-stage larva, the male was practically indistinguishable from the female in this stage prior to the fourth moult. They had the same general body form, with the exception of an expansion at the position of the rectal area in the case of the male, the same sclerotized cephalic framework, strong stylet, etc. Not until the fourth molt did drastic changes take place in the male. During this molt it did not increase in length, it lost its sclerotized cephalic framework; the head became set off and was not reinforced; the strong larval stylet was replaced by a very weak, probably

not functional substitute; the median bulb was small and not muscular and the esophageal glands remained underdeveloped.

Adults. — The nemas emerged from the fourth molt as young adults with their reproductive organs fully formed. In females, however, growth in size and especially development of the gonads continued. The anterior ovaries ranged from 110-226 μ and were usually longer than the posterior ones, which ranged from 105-270 μ in length. Male gonads ranged from 168-223 μ. A long developed gonad of an older female is shown in Figure 4, L. The various measurements for adult burrowing nemas have been presented earlier (VAN WEERDT, 1958).

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ZUSAMMENFASSUNG

Studien zur Biologie von Radopholus similis *(Cobb, 1893) Thorne, 1949. 3. Teil: Embryologie und postembryonale Entwicklung*

Embryonal- und Postembryonalentwicklung von *Radopholus similis* werden beschrieben und abgebildet. Die Lage der Richtkörper am vorderen Pol dient der Orientierung der Blastomeren. Die Embryonalentwicklung ist temperaturabhängig; sie dauert 4-10 Tage. Die erste Häutung findet vor dem Schlüpfen statt. Drei weitere Häutungen erfolgen nach dem Schlüpfen. Der Versteifungsapparat, die Cheilorhabdions, die Prorhabdions, die Abkleidung der beiden amphidialen Gefäße des terminalen Exkretionsgefäßes und des Rektums samt Kutikula werden während der Häutung abgestoßen. Unterschiede zwischen der Entwicklung des männlichen und des weiblichen Genital-Primordiums erlauben die Diagnose von Männchen und Weibchen schon im zweiten Stadium.

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GIANT LARVAE OF THE CLOVER CYST-NEMATODE *HETERODERA TRIFOLII* (NEMATODA: HETERODERIDAE)

BY

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MULVEY (1958b, 1959b) reported impregnation of females of the clover cyst-nematode *Heterodera trifolii* Goffart, by males of the sugar-beet nematode *H. schachtii* Schmidt , and found that a number of giant eggs were produced by the second generation females which originated from a mixed population of this parthenogenetic species and *H. schachtii*. ONIONS (1953) described four giant larvae which had emerged from a single cyst of the potato-root eelworm *H. rostochiensis* Wollenweber. He suggested that either gene mutation or polyploidy was responsible for their formation. The following is a report on the production of giant larvae from second generation females of *H. trifolii* which had originated from a mixed population of this species and *H. schachtii*.

MATERIALS AND METHODS

Second generation females of the clover cyst-nematode were obtained by partly filling a box with a mixture of sterilized sand and soil compost heavily infested with the clover cyst-nematode derived from a single-cyst culture. After a few days white Dutch clover seedlings were transplanted into this box. A second box was partly filled with sterilized compost heavily infested with the sugar-beet nematode, *H. schachtii,* also derived from a single-cyst culture. A few days later red beet seedlings were transplanted into this box. After two days, during which time the second-stage larvae had entered the plant roots, the seedlings were removed from both boxes, washed free of debris and one plant of each kind was replanted in each of twenty 5-inch pots of sterilized compost.

Thirty-five days after replanting the white Dutch clover plants were removed from the soil, and carefully washed before examination by immersing it in water. Gravid white females were removed and white Dutch clover seedlings, growing in 5-inch pots containing sterilized soil, were inoculated with these first generation females. Thirty-five days later these plants were removed from the pots, the roots washed free of debris, and the second generation females removed from the white Dutch clover roots and then placed in petri dishes containing a little water. The larvae, which eventually hatched from eggs within these females, were carefully checked for giant forms. The giant larvae, when found, were removed from the

water and relaxed by heat. Temporary water mounts were made of both giant and normal larvae. These were examined under the microscope and dimensions determined from camera lucida drawings of each specimen.

DIMENSIONS

TABLE I

Dimensions of giant and normal larvae of H. trifolii *from the same population*

Number of larvae	Average length body (μ)	stylet (μ)	esophagus (μ)	tail (μ)
Giant — 11	675 (630-760)	32 (31-35)	230	160
Normal — 25	503 (450-520)	27.7 (27-29)	150	65

There was no overlap in body length between giant (Fig. 1, A) and normal larvae (Fig. 1, B).

ANATOMICAL DIFFERENCES

The stylet shaft (Fig. 1, C) in the giant form is much stouter and the stylet knobs heavier than those of the normal larva (Fig. 1, D). In a few of the giant larvae the stylet knobs project forward very distinctly. The tail termins in the giant form is generally bluntly rounded and the germinal primordium larger than that of the normal larva.

DISCUSSION

Only eleven giant larvae were found in approximately 7000 larvae examined However, examination of gravid females for egg size showed fifteen to twenty contained from one to four giant unhatched eggs.

H. trifolii is a parthenogenetic nematode of the diploid type (MULVEY, 1958a). At first metaphase of the maturation division it has twenty seven chromosomes (MULVEY, 1959b) which indicates that it is a triploid by comparison with other species of cyst-forming nematodes which have been examined for chromosome number. According to MULVEY (1957; 1959a) *H. schachtii* and *H. avenae* both have a haploid number of nine chromosomes. RILEY & CHAPMAN (1957) reported the same number for *H. rostochiensis*.

The giant larvae of *H. trifolii* may have been produced by the sperm of *H. schachtii* entering the *H. trifolii* oocytes of the first generation females. Union of male pronucleus (nine chomosomes) and female pronucleus (twenty-seven chromosomes) may have resulted in the production of giant tetraploid larvae with thirty-six chomosomes.

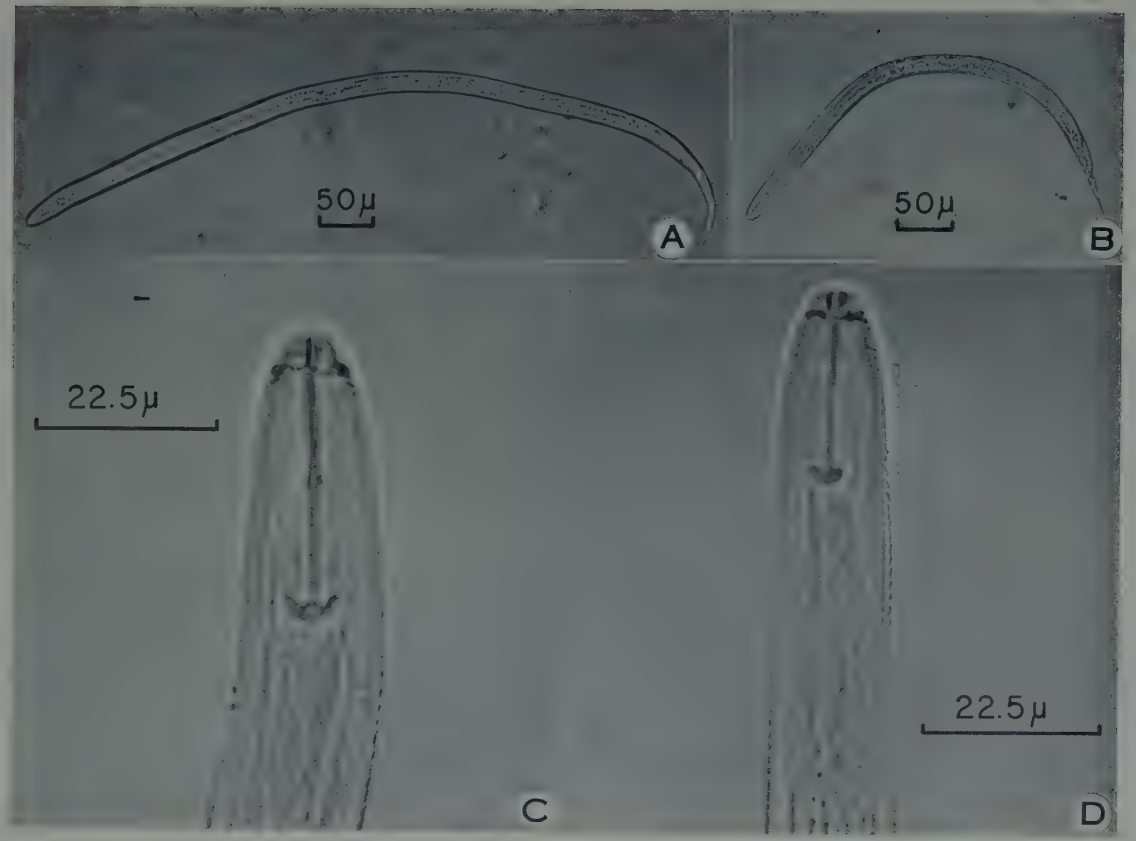

Fig. 1. Photomicrographs of second-stage larvae of *Heterodera trifolii*. A, Giant larva; B, Normal larva; C, Head end of giant larva; D, Head end of normal larva.

The course of events may be as follows:

Generation 1

H. schachtii		*H. trifolii*
(Adult males containing sperm having nine chromosomes)	×	(Females containing oocytes with twenty seven chromosomes)

Some *H. trifolii* females are impregnated with sperm of *H. schachtii* males. Sperm enters many oocytes.

Generation 2

H. trifolii larvae produced by generation 1 females develop into generation 2 females. These produce normal larvae and a few giant larvae which are probably tetraploids.

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ZUSAMMENFASSUNG

Riesenlarven des Kleezystennematoden, Heterodera trifolii (*Nematoda*: *Heteroderidae*)

Weibchen der zweiten Generation des Kleezystennematoden, *Heterodera trifolii*, die aus Mischkulturen der parthenogenetischen Art und des Rübennematoden, *Heterodera schachtii*, stammten, erzeugten mehrere Riesenlarven in einer Population von einigen tausend normalen Larven. Die Riesenlarven hatten eine Körperlänge von 675 μ und eine durchschnittliche Stachellänge von 32 μ. Die Larven sind wahrscheinlich tetraploid.

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THE SENSITIVITY OF SOME PLANT-PARASITIC AND FREE-LIVING NEMATODES TO GAMMA AND X-IRRADIATION

BY

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Little has been published on the effects of ionizing radiation on plant-parasitic and free-living nematodes. COBB (1920) reported that exposure of *Heterodera radicicola* (*Meloidogyne* spp.) to an unspecified dose of X-rays had no observable effects since root galls developed normally. Populations of *Ditylenchus destructor* and *Rhabditis* sp. were reduced by over 98 % after exposure to 48,000 roentgens (r.) and reproduction failed after 96,000 r. (WOOD & GOODEY, 1957). *Rhabditis pellio* irradiated with 4928 r. produced 2.5 offspring per female, whereas unirradiated females produced 86.5 young (EVANS, LEVIN & SULKIN, 1941). FASSULIOTIS (1958) found great variation in the proportion of viable second-generation eggs in cysts of *Heterodera rostochiensis* after irradiation with 20,000 r., and sterilization occurred at doses of 40,000 to 80,000 r. A significant decrease in hatch was obtained from exposure to 160,000 r. Delayed emergence, reduced infectivity, altered motility, and chromosome damage were also noted. Similar but less detailed work was done on *Heterodera rostochiensis* by WEISCHER (1957).

This paper presents data on doses of radiation which caused sterilization in fourteen of free-living and plant parasitic nematodes. The doses causing death, temporary sterilization, embryo destruction, altered movement, variation in length and other morphological changes are reported for one or more species. The impracticability of using radiation for control of nematodes was previously reported (MYERS & DROPKIN, 1959).

METHODS

Inoculum consisting of nematode-infested soil and chopped roots was irradiated by cobalt-60 sources in 1-$^3/_4$ inch diameter polyethylene plastic tubes. The dose rates used ranged from 200,000 to 670,000 r./hr. and the dosages received by the samples ranged from 20,000 to 500,000 r. with a possible error of $\pm$ 10 %. The irradiated inoculum was then potted with appropriate host plants. A typical experiment consisted of uninoculated pots, inoculated unirradiated pots, and pots with inoculum irradiated at 40,000, 80,000, 160,000, and 320,000 r. replicated

three times. Standard techniques of nematode collection and population counting were used to assess the results of the treatments.

Two species of non-parasitic nematodes were cultured in the laboratory. *Turbatrix aceti* was cultured in 50 ml. flasks of diluted apple vinegar. Unirradiated controls and nematodes irradiated in water at doses of 20,000, 40,000, 60,000, and 80,000 r. from cobalt-60 were set up in two-fold replication. *Panagrellus silusiae* was cultured on medium containing peptone, dextrose, beef extract, yeast extracts, gelatin, antibiotics, and water. Nematodes were irradiated in water and two replications were set up consisting of controls and irradiation doses of 20,000, 30,000, 40,000, 50,000, and 60,000 r. In a similar experiment, *Panagrellus silusiae* was exposed to X-rays at doses of 20,000, 40,000, and 60,000 r. A dose rate of 1,465 r. per minute, at 250 KV., 30 MA., with a 1 mm aluminium filter, was used in this and all subsequent experiments with X-rays.

Measurements of nematodes were made from camera lucida drawings.

STERILIZATION BY IRRADIATION

Table I shows the minimum dose which prevented reproduction and the maximum dose where reproduction occurred. The doses required to give complete sterilization were never less than 20,000 r. and some were higher than 160,000 r. Reduction in populations occurred at doses below those required for complete sterilization. Five months after irradiation with 40,000 r., the population of *Helicotylenchus nannus* was 100 % of the controls; *Scutellonema brachyurum,* 100 %; *Mononchus* sp., 95 %; *Helicotylenchus buxophilus,* 50 %; *Xiphinema diversicaudatum,* 20 %; *Dorylaimus* sp., 10 % and *Criconemoides axeste,* 10 %.

Many different effects of irradiation were observed. *Helicotylenchus nannus* showed abnormally large, partially developed eggs and vacuolization of the gonads after 80,000 r. and above. *Trichodorus christiei,* following irradiation with 40,000 r. and above, was larger in size than the controls. The population at 5 months had increased by five times after a dose of 40,000 r., by three times after 80,000 r., and the population exposed to 160,000 r. was slightly less than the control. More *Tylenchorhynchus claytoni* died as the dose increased from 200,000 to 500,000. The population of the unirradiated control 2 months after the start of the experiment was 23,670 nematodes, whereas populations irradiated with 200,000, 300,000, 400,000 and 500,000 r. contained 2,170, 1,860, 850 and 60 nematodes respectively. Gonad formation occurred up to 400,000 r. but granulation in the intestine obscured observation of gonads after exposure at 500,000 r. No males were found in the mass population flasks of *Turbatrix aceti* 50 days following irradiation with 40,000 r. and above. *Panagrellus silusiae* was sterilized at approximately the same dose by both X-rays and cobalt-60 rays as indicated by small populations in the 40,000 r. cultures. Reproduction was interrupted and embryos were damaged at all doses, and no larval forms were found in the cultures 20 days after exposure to 60,000 r.

TABLE I

Sensitivity of plant-parasitic and free-living nematodes to cobalt-60 radiations

Nematode species	Dose rate 1×10^3 r./hour	Reproduction	
		Some 1×10^3 r.	None 1×10^3 r.
Aphelenchoides sp.	269	0	40
Pratylenchus vulnus Allen and Jensen, 1951	300	0	40
Heterodera rostochiensis Wollenweber, 1923	535	20 *	—
Turbatrix aceti (Müller, 1783) Peters, 1927	500	20	40
Panagrellus silusiae (Co-60)	200	40	50
De Man, 1913 (X-ray)	87.9	40	60
Xiphinema diversicaudatum (Micoletsky, 1927) Thorne, 1939	300	40	80
Mononchus sp.	269	40	80
Tylenchorhynchus claytoni Steiner, 1937	260	60	80
Criconemoides axeste Fassuliotis & Williamson, 1959	300	80	160
Dorylaimus sp.	269	80	160
Helicotylenchus buxophilus (Golden, 1956) Perry, 1959	269	160	320
Helicotylenchus nannus Steiner 1945	269	160	320
Scutellonema brachyurum (Steiner, 1938) Andrassy, 1958	310	160	320
Trichodorus christiei Allen, 1957	269	160	320

* Less than 1 % reproduction.

INCREASED LENGTHS AFTER EXPOSURE TO RADIATION

Some nematodes extracted from the sterilization trials appeared larger than normal, so detailed observations were made on two species. Measurements of *Tylenchorhynchus claytoni* 42 days after irradiation compared with controls indicated that no significant differences existed in the length of the stylet or oesophagus, position of the vulva, or the ratios of total length to the greatest width (a), of total length to the length of the oesophagus (b), and of total length to the length of the tail (c), however, total length differed (Table II). The largest males and females of *Turbatrix aceti* were selected from unirradiated cultures for comparison with nematodes randomly selected 50 days after irradiation (Table II). Since increased length seemed related to sterilization, isolations of *Turbatrix*

aceti were made to study the effects of lack of reproduction without irradiation. One hunderd young larvae were placed individually in plastic vials filled with vinegar and measurements made after 53 days (Table II). The lengths of isolated

TABLE II

Lenghts of adult nematodes after irradiation *

Species	Unirradiated Control	Amount of radiation 20,000 r.	40,000 r.	60,000 r.	80,000 r.	120,000 r.
Tylenchorhynchus claytoni						
Male	640 ± 10	———	670 ± 10	———	650 ± 10	660 ± 20
Female	620 ± 20	———	720 ± 10	———	730 ± 20	720 ± 10
Turbatrix aceti						
Male	1222 ± 21	1296 ± 49	———	———	———	———
Female	1302 ± 28	1785 ± 24	1768 ± 35	1795 ± 46	1789 ± 50	———
Turbatrix aceti (isolated)						
Male	1315 ± 24	———	———	———	———	———
Female	1763 ± 39	———	———	———	———	———

* Mean of 10 measurements ± standard error of mean (Microns).

Turbatrix aceti were similar to those of the irradiated nematodes. Some two-celled ova occurred in the isolated females although no larvae were produced. The males disappeared from the mass cultures irradiated at 40,000 r. or more, but survived in isolation. In all cases female length differences were above the 95 % confidence level, whereas male lengths were not significantly different when compared with the unirradiated controls.

EFFECTS OR IRRADIATION ON THE LIFE CYCLE OF *Panagrellus silusiae*

Unirradiated larvae of *Panagrellus silusiae* increased rapidly in length on the gelatin medium. The length of the larvae at 1 day was 0.25 mm, at 2 days 0.47 mm, and at 3 days the length varied from 0.45 mm to 0.75 mm. The nematodes were mature by the sixth day and the lengths of the male and female nematodes averaged 1.0 mm and 1.4 mm, respectively. Single females isolated before copulation produced no progeny, but when males were included an average of 84.6 (± 10) larvae per female were produced. Copulation sometimes occurred more than once. Young were born 20 hours after fertilization. The period from insemination to death averaged 11 days, thus making the total life span for the females 17 days. Males lived slightly longer.

Embryos in the uterus of *Panagrellus silusiae* rapidly became disorganized after irradiation from cobalt-60 at doses of 10,000 to 60,000 r. The reproductive tracts

were filled with the remains of disintegrated embryonic material and some debris was ejected from the vulvas. Eight days after irradiation with 10,000 and 20,000 r., embryonic debris was observed followed by live larvae and apparently normal embryos. A small number of larvae were produced 10 days after irradiation with 40,000 r. Doses of 60,000 r. completely sterilized *Panagrellus silusiae* and some adults were found with irregularly formed gonads after 40,000 r.

Larvae exposed 60,000 r. before the end of their first day were stunted. The average length of 10 irradiated larvae was only 0.42 mm after 10 days or slightly less than that of the average length of 2-day old larvae. The oesophageal bulbs appeared misshapen, and anterior oesophageal constrictions were sometimes found. Some larvae kept beyond 10 days developed into adult forms but produced no young.

SENSITIVITY OF EMBRYOS TO RADIATION

Although completely developed larvae of *Panagrellus silusiae* in the reproductive tract of 8-day old females were born living after irradiation with 60,000 r. (X-rays), few embryos finished development. These females contained 8 to 25 embryos in all stages of development of which stages from gastrulation and beyond accounted for 32 %. Counts of the number of living larvae developing from irradiated embryos (Table III) as compared with the embryonic stages remaining within the reproductive tracts indicated resistance to the effects of X-rays

TABLE III

Percentage of embryonic stages of Panagrellus silusiae *developing into living larvae after X-radiation*

Dose roentgens	No. of Replications 10 females/replication	Percentages
Unirradiated Control	26	100
2,500	13	88 ± 3
5,000	12	60 ± 6
7,500	11	45 ± 3
10,000	12	36 ± 6
12,500	13	37 ± 6
15,000	13	34 ± 6

increased with development and that embryos past gastrulation developed into living larvae even after exposure to 15,000 r.

INJURY AND DEATH CAUSED BY HIGH DOSES OF RADIATION

Over 99 % of *Panagrellus silusiae* were killed within 24 hours following a dose of 1,500,000 r. from cobalt-60, whereas after 960,000 r., the population was still approximately 5 % of the unirradiated control. Abnormal movements

such as swimming backwards were noted. The criterion for death was lack of movement; but during counting, the numbers of nematodes recorded as living increased with the length of the observation period.

The rapid movement of *Panagrellus silusiae* appeared normal up to a dose of 200,000 r., and then as the doses increased, the movements progressively slowed down and became jerky. Few nematodes had normal movement after irradiation at doses between 400,000 and 500,000 r. Motility ceased following a dose of 500,000 r. However, the nematodes were not dead since a slow light movement could still be noted in the head region. These movements occurred between periods of rest that became longer with increased dose. Apparently there was some recovery because over 85 % of the nematodes were motionless 24 hours after irradiation with 500,000 r., but 72 hours after irradiation with a dose of 800,000 r. only 24 % were motionless.

DISCUSSION

Gomberg & Gould (1953), Gould, van Dyke, & Gomberg (1953), found that *Trichinella spiralis* required at least 750,000 r. to kill larvae *in vivo* and *in vitro*. Fassuliotis (1958) irradiated the cysts of *Heterodera rostochiensis* and showed that no hatch occurred after a dose of 640,000 r. Dropkin (in litt.) established that eggs of *Meloidogyne arenaria* ssp. *thamesi* and *Meloidogyne incognita* v. *acrita* hatched in reduced numbers following irradiation with 360,000 r.; after 720,000 r. no further hatch occurred. These data are in general agreement with the findings of this study. A few *Panagrellus silusiae* were found to survive doses up to 1,500,000 r. for 24 hours; some survived for 3 weeks in culture after a dose of 960,000 r. Some *Tylenchorhynchus claytoni* survived 500,000 r. for 2 months. Most species survived 2 months without serious decrease in population at doses up to 320,000 r. It seems likely that species that did not survive two months after irradiation with 320.000 r. have a life span shorter than this period.

Populations were found reduced at doses far less than that required for sterilization. Temporary sterilization occurred in *Panagrellus silusiae* and apparently normal embryogenesis resumed after a sufficient period had elapsed. The movement of *Panagrellus silusiae* was progressively retarded with doses above 200,000 r.

The embryonic stages previous to gastrulation were highly sensitive to radiation damage. Development of *Panagrellus silusiae* embryos was interrupted and an unorganized mass of cells resulted after doses under 10,000 r., yet from 10,000 to 15,000 r. no further increase in damage was noted and the nematodes past the gastrulation stage developed into larvae. Radiation resistance after gastrulation seems related with the fact that all the basic tissue lines are essentially complete and few cell divisions are necessary to complete larval development. These data agree in principle with those of Seide (1925), who showed that 99 % abnormalities occurred after X-ray irradiation of one-celled *Ascaris* eggs, 24 % abnormal-

ities of 4-celled eggs; the blastula stage showed 5 % damage, and the gastrula stage 4 %, whereas the larvae showed 2.5 % damage. The controls had 1 % of abnormalities. This dose was calculated to be about 2,000 r. PERTHES (1904) mentioned that the later stages of embryonic development were more resistant to irradiation, but ZUPPINGER (1928) claimed a rise in sensitivity at gastrulation of *Ascaris* embryos.

Unless some severe physical damage occurred, irradiated larvae became adults. Cell maturation occurred after all doses up to 500,000 r. as indicated by elongation of the body and by the formation of the vulva and the spicules. Gonad formation occurred in all genera following irradiation up to 320,000 r. and in *Tylenchorhynchus claytoni* up to least 400,000 r. Doses above 400,000 r. prevented accurate observation since vacuolization and granulation occurred in the intestinal region. Gonad formation was interrupted in but few nematodes and when this occurred it was probably caused by the irradiation of cells undergoing division. Thus, interphase cells which constitute all cells except those of the gonad appeared essentially unaffected by radiations at the doses used.

Morphological changes occurred when young larvae were irradiated and allowed to elongate. These changes were represented by thickenings and nodulations of the cuticle and were found in most species. Intestinal darkening, clearing of intestine, patchiness with light and dark areas, and granulation were noted. This type of damage was noted by other authors (THOMAS & QUASTLER, 1950; BABERO, 1952).

Some reduction in size occurred in *Panagrellus silusiae* after irradiation of larvae less than 1 day old, but this was caused by injury to the oesophagus. Growth occurred in some larvae after 10 days. Decreased length was noted by GOULD, GOMBERG, BETHELL, VILLELLA & HERTZ (1955). Size increases were mentioned by TYZZER & HONEIJ (1916) and BABERO (1952), but measurements were not made. Size increases seem to be correlated with sterilization or lack of reproduction, since increase in length of isolated, unirradiated nematodes was about equal to that of nematodes sterilized by irradiation.

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ZUSAMMENFASSUNG

Die Empfindlichkeit einiger pflanzenparasitärer und freilebender Nematoden gegenüber Gamma- und Röntgenstrahlen

Die Sterilisationswirkung ionisierender Strahlen wurde an 14 Nematodenarten untersucht. Populationsverminderungen konnten mit wesentlich geringeren Strahlendosen erzielt werden, als sie für eine vollständige Sterilisation erforderlich waren. Das empfindlichste Stadium im Entwicklungszyklus

von *Panagrellus silusiae* war der Embryonalzustand. Larven und erwachsene Tiere vertrugen sehr hohe Strahlendosen. Zellteilungen fanden bei den meisten Arten bis zu 320 000 statt, sofern die Gonaden gebildet waren. Bei *Tylenchorhynchus claytoni* trat Zellreifung bis zu 500 000 r auf, wie sich am Längenwachstum und an der Bildung von Vulva und Spicula zeigte. An Körpergrössenänderungen waren sowohl Erhöhung als auch Verminderung der Körperlänge als Folge der Bestrahlung zu beobachten. Diese beiden entgegengesetzten Wirkungen gingen einmal zurück auf Schädigungen, die die Nahrungsaufnahme verhindern und zum anderen auf eine Sterilisation ohne Beeinflussung der Nahrungsaufnahme. Morphologische Veränderungen wurden bei allen Arten beobachtet und umfassen kutikulare und innere Schäden.

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THREE ADDITIONAL SPECIES IN THE GENUS *HEMICRICONEMOIDES* CHITWOOD AND BIRCHFIELD 1957 (NEMATA: TYLENCHIDA)

BY

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In February 1958 *Camellia* sp. plants exhibiting symptoms of root injury were submitted to the State Plant Board Laboratory. Subsequent examination of the soil associated with the roots revealed the presence of a previously undescribed species of *Hemicriconemoides* here designated as *H. chitwoodi* n. sp. In July 1958 while investigating the nemic population associated with the tap roots of shade trees, a second previously undescribed species was recovered from the soil about the deep roots of *Carya glabra* var. *megacarpa* Sarg. This species here designated as *H. minutus* n. sp. was recovered at a later date from soil collected from about the roots of *Rhododendron indica* Sweet, *Pinus elliottii* Engelmann, and *Citrus* sp. A third previously undescribed species of *Hemicriconemoides* here designated as *H. strictathecatus* n. sp. was recovered from soil about the roots of *Cocus nucifera* L.

Hemicriconemoides chitwoodi n. sp.
(Fig.1-A, B, C, D, E)

13 ♀♀ Length 540 (480-590) microns
a = 15.5 (13.4-17.0), b = 3.8 (3.5-4.1), c = 15.7 (11.2-21.7)
V % = 90.1(88.1-91.0)
Stylet length 91 μ (85-95). Sheath annules 124 (116-133)
2 ♂♂ Length 400-424 μ. a = 25.6-26.6, b = ?, c = 13.6-14.6
Female (*Holotype*) Length 480 microns; a = 15.2; b = 3.6; c = 13.3;
V = 91 %. Stylet 92 μ; Annules 121.
Male (*Allotype*) Length 424 microns; a = 26.6 μ; b = ?; c = 14.6; T = 37 %.

Female description: Cephalic region with 2 annules, the second annule moderately constricted, not as wide as adjacent annule. Tail conoid, almost twice the anal body diameter in length. Annules about 4.5 μ wide. Lateral fields absent. Cervical papillae, cephalids, phasmids and hemizonid not observed. Cheilostom 3.5 μ long. Stylet well developed, prorhabdions 77 μ long, meso-metarhabdions 4-5 μ long.

Fig. 1. — *Hemicriconemoides chitwoodi* (A, B, C, D, E).
A. Male. B. Female. C. Female, posterior extremities. D. Female esophageal region. E. Cuticle detail.
Hemicriconemoides minutus (F, G, H).
F. Female esophageal region. G. Female posterior extremities. H. Female.

Telorhabdions 3.6 μ long by 8.0 μ wide. Stylet knobs sometimes directed anteriad, otherwise rounded. Dorsal gland orifice 4.5 μ posterior to base of telostom. Metacorpus and posterior esophageal bulb typical of genus. Intestine overlaps the posterior esophageal bulb slightly. Excretory pore located 140 μ from anterior end of body. Anus located on 9th annule anterior to terminus. Visible portion of rectum appears as a yellow refractive band 1 μ wide by 4-6 μ long. Vulva located between 12th and 15th annule from terminus. Gonad prodelphic extending slightly anterior to meso-metarhabdions. Spermatheca round to slightly oval. Sperm in spermatheca small and rounded similar to sperm in vas deferens of male.

Male description: Cephalic region continuous with body. Sheath and stylet absent. Esophageal region indistinct, with no clear demarcation between esophagus and intestine. Caudal alae absent. Tail conoid ending in a sharp point. Three lateral fields present. They originate on the 3rd annule posterior to the head as a single row of blocks which gradually widen for about ¼ of the body length after which a third lateral line appears. The two rows of blocks continue for a short distance, then subdivide into four lateral lines without transverse markings that extend to the 6th annule from the terminus. A single gonad extends anteriad 128 μ from the cloacal opening. Spicule 29.1 μ long. Slightly arcuate.

Holotype: Female collected February 5, 1958, by K. P. Bragdon and R. H. Miller, Slide No. H-47, State Plant Board of Florida Nematology Collection.

Type Habitat: Soil about the roots of *Camellia* sp.

Type Locality: Monticello, Florida.

Diagnosis: *Hemicriconemoides chitwoodi* most closely resembles *H. gaddi* (Loos, 1959) Chitwood & Birchfield, 1957. Females of *H. chitwoodi* have longer stylets with more rounded knobs than *H. gaddi*. The male of *H. chitwoodi* is longer than the male of *H. gaddi* and has two more lateral lines.

Hemicriconemoides minutus n. sp.
(Fig. 1-F, G, H)

11 ♀♀ Length 360 (330-395) microns. a = 12.7 (11.3-13.7), b = 3.2 (2.9-3.8), c = 14.2, V % = 94.0 (92.0-96.0)
Stylet length 75 μ (72-76), sheath annules 88 (86-94)

Female (*Holotype*) Length 347 microns; a = 12.8; b = 3; c = ?; V = 94.2 %. Stylet 76 μ. Annules 91.

Female description: Cephalic region not set off. Tail rounded. Annules 4.6 μ wide, lateral fields absent. Cervical papillae, phasmids, and hemizonid not seen. Stylet well developed, prorhabdions 69.3 μ long, meso-metarhabdions 7 μ long, telorhabdions variable from one knob hooked strongly anterior to all knobs projecting slightly anteriad, 4.2 μ long by 8 μ wide. Dorsal gland orifice 5 μ posterior to base of telorhabdions. Excretory pore 109 μ from anterior end. Intestine overlaps posterior esophageal bulb slightly. Rectum obscure, anus 25 μ from tail tip. Vulva between 6th and 9th annule anterior to terminus. Gonad

prodelphic, extending to the valves in the metacorpus. Oviduct dilated at its junction with the uterus. A spherical spermatheca lies within this dilation.

Male and larvae not observed.

Although males were not recovered, the similarity in form of the sperm and spermatheca of *H. minutus* to those of *H. chitwoodi* suggests the existence of males.

Holotype: Female collected July 28, 1958 by R. P. Esser. Slide No. H-203, State Plant Board of Florida Nematology Collection.

Type Habitat: Soil about deep roots of *Carya glabra* var. *megacarpa* Sarg.

Type locality: Southeast Campus, University of Florida, Gainesville, Florida.

Diagnosis: *Hemicriconemoides minutus* is most closely related to *H. brachyurus* (Loos, 1949), Chitwood & Birchfield, 1957 and *H. strictathecatus,* both of which have rounded tails. *H. minutus* females have longer stylets and fewer sheath annules than *H. brachyurus*. *H. minutus* females differ from *H. strictathecatus* females in possessing fewer sheath annules, anteriorly hooked rather than spherical stylet knobs and being smaller in size.

Hemicriconemoides strictathecatus n. sp.

(Fig. 2-A-G)

10 ♀♀ Length 560 (510-590) microns. a = 17.8 (15.6-19.0)
b = 4.4 (4.1-4.7), c = 17.5 (16.0-19.3), V % = 92.7 (91.3-93.2)
Stylet length 79 μ (73-83). Sheath annules 138 (127-152)

2 larvae. Length 318, 355 microns. a = 12.2, 13.2, b = 2.7, c = ?
Stylet 75 μ, 79 μ.

Female (*Holotype*) Length 554 microns; a = 17.5; b = 4.7; c = 18.4;
V = 92.7 %. Stylet 78 μ; Annules 136.

Female description: Cephalic region comprised of a single, well-set-off, disc-shaped annule. Tail rounded to bluntly conoid. Annules 5.3 μ wide. Lateral fields absent. Cervical papillae, cephalids, phasmids and hemizonid not seen. Prorhabdions 60 μ long, meso-metarhabdions 10 μ long, telorhabdions 5.2 μ long by 8 μ wide. Dorsal gland orifice 5 μ posterior to telostom. Metacorpus and posterior esophageal bulb typical of genus. Intestine rarely overlapping posterior esophageal bulb. Position of excretory pore varied from 103 μ to 155 μ from anterior end. Visible portion of rectum appears as a wide band 1.6 μ wide by 3 μ long on 9th annule from terminus. Vulva between 11th and 13th annules from terminus. Gonad prodelphic, extending to metacorpus valve. A large spermatheca, 18.9 μ long by 24.1 μ wide located within the oviduct near its junction with the uterus. Egg 65 μ long by 18 μ wide.

Larval description: Head set off by a single disc-like annule. Tail conoid. Sheath absent. Cuticle ornamented with 12 rows of conoid spines. Stylet similar to that of adult female. Excretory pore 110 μ from anterior end. Rectum or anus not observed.

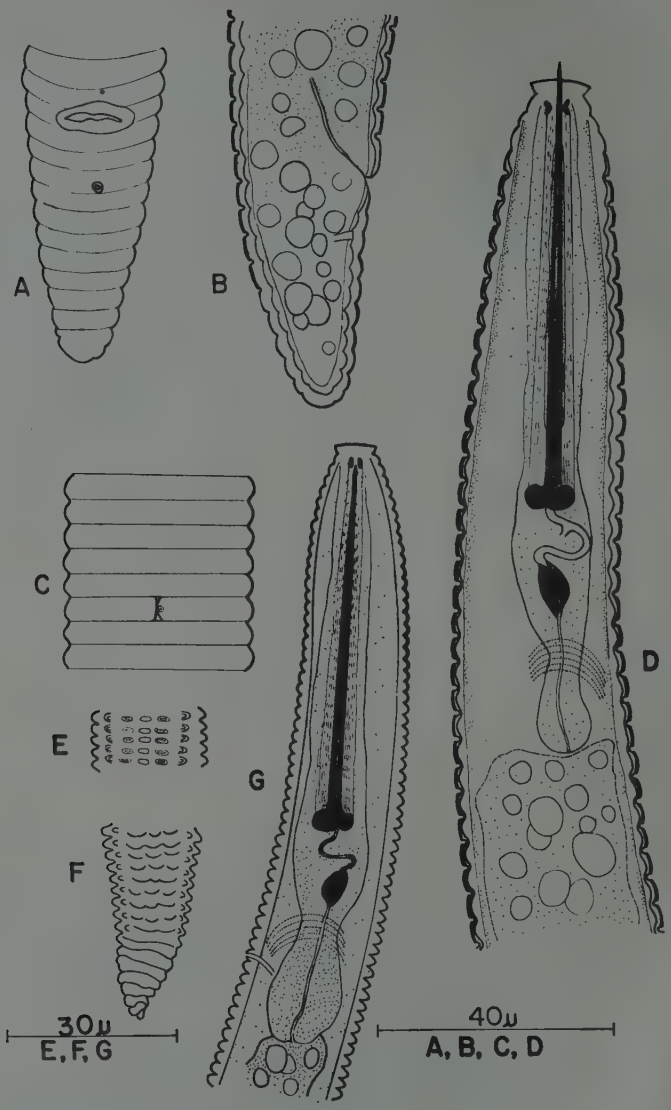

Fig. 2. — *Hemicriconemoides strictathecatus.*
A. Female tail, ventral view. B. Female tail, lateral view. C. Female, ventral view showing annule break above excretory pore. D. Female, esophageal region. E. Appearance of larval spines at mid-body. Left, lateral view. F. Tail of larva. Left, lateral view. G. Larval esophageal region. Left, lateral view.

Holotype: Female collected Frebruary 20, 1959 by L. van Weerdt and A. Martinez. Slide Number H-732, State Plant Board of Florida Nematology Collection.

Type Habitat: Soil about the roots of *Cocos nucifera* L.

Type locality: Key West, Florida.

Diagnosis: *Hemicriconemoides strictathecatus* differs from all other species in the genus by possessing spheroid stylet knobs and having its cephalic region set off by a single disc-shaped annule.

MORPHOLOGICAL OBSERVATIONS

The sheath was found to be attached to the body at the vulva, the excretory pore and high on the first head annule. Several specimens of *H. chitwoodi* were seen with the sheath separated anteriorly.

This type of exsheathment may lend support to the hypothesis (ROGERS 1957) that molting is controlled by endocrine systems releasing exsheathment fluid in the esophageal region, causing a fracture in the sheath in this region. This same phenomenon was recently observed in *Xiphinema* sp. and *Helicotylenchus* sp. females. These females were seen emerging from fourth-stage larval cuticles, from a fracture at the level of the nerve ring. Similar observations have been made with *Radopholus similis* (VAN WEERDT, in litt).

One specimen of *H. strictathecatus* viewed ventrally exhibited a single annule break in the sheath directly over the excretory pore (Fig. 2-C). Subsequent examinations, however, failed to reveal the same annule break in other specimens.

In *H. minutus* it was established that the number of body annules equals the number of sheath annules. Flattening of the sheath annules in the subfamily Criconematinae (Fig. 1-E) was thought to be peculiar to the genus *Hemicriconemoides* (CHITWOOD & BIRCHFIELD 1957) until the same annule flattening was observed in two recently described species in the genus *Hemicycliophora, H. tesselata* (SAUER 1958) and *H. brevicauda* (SAUER 1958).

Hemicriconemoides strictathecatus has the snuggest sheath of all observed species within the genus. In some specimens the sheath separation is seen only by critical examination under an oil immersion objective. *Hemicriconemoides* appears to have a closer affinity to *Criconemoides* than to *Hemicycliophora*. Evidence suggesting this is the reduction in number of body annules, lack of caudal alae, and the similarity in male spicules between *Hemicriconemoides* and *Criconemoides*. Further support was expressed for this affinity by the larvae of *H. strictathecatus* both in spination and lack of a sheath. Unfortunately males and larvae have not been described for many species in the three aforementioned genera, so no broad statements can be made at this time.

Three specimens of *H. strictathecatus* seen with various degrees of stylet extension provoked the following questions: Does the esophageal lining between the telorhabdions and the metacorpus with its convolutions limit stylet extrusion? Does the lumen stretch, or can the valve, bulb and glands be pulled forward? In

an attempt to answer these questions, the following measurements were made with the three specimens in 2 percent formalin shortly after cessation of movement (Table I):

TABLE I

Specimen	A	B	C	D	Body length	Stylet
1	11 μ	Convoluted	86 μ	122 μ	570 μ	80 μ
2	32 μ	24 μ	73 μ	106 μ	580 μ	79 μ
3	52 μ	26 μ	57 μ	?	590 μ	79 μ

A = length of extruded stylet (anterior to anterior end of nema).
B = length of esophageal lining between telorhabdions and metacorpus valve.
C = distance from metacorpus valve to anterior end of nema.
D = distance from base of esophageal bulb to anterior end of nema.

The results, based on these limited observation, indicate flexibility in the esophageal organs. As a result of stylet extrusion, both the esophageal bulb and metacorpus were pulled forward.

TABLE II

Geographical distribution and host plant association of various species of Hemicriconemoides *as indicated (Collected from soil closely associated with roots, host status unproven.)*

Species	Locality	Probable host plant
Hemicriconemoides chitwoodi	Citra, Florida	*Cycas* sp.
Hemicriconemoides chitwoodi	Citra, Florida	*Wisteria* sp.
Hemicriconemoides cocophilus	Monticello, Fla.	*Stenotaphrum secundatum* Kuntze
Hemicriconemoides cocophilus	Jamaica, B.W.I.	*Cocos nucifera* L.
Hemicriconemoides cocophilus	Key West, Fla.	*Cocos nucifera* L.
Hemicriconemoides wessoni	Key West, Fla.	*Cocos nucifera* L.
Hemicriconemoides wessoni	Sanford, Fla.	*Arecastrum romanzoffianum* Becc.
Hemicriconemoides wessoni	Deland, Fla.	*Cynodon dactylon* Pers.
Hemicriconemoides wessoni	Jacksonville, Fla.	*Magnolia* sp.
Hemicriconemoides wessoni	Baldwin, Fla.	*Pinus palustris* Mill.
Hemicriconemoides wessoni	Lake Monroe, Fla.	*Podocarpus macrophylla,* D. Don
Hemicriconemoides wessoni	Gainesville, Fla.	*Ulmus floridana*
Hemicriconemoides wessoni	Monticello, Fla.	*Zoisia*
Hemicriconemoides strictathecatus	Jamaica	*Cocos nucifera* L.

Acknowledgment is made to Dr. B. G. Chitwood for excellent guidance and instruction.

ZUSAMMENFASSUNG

Drei neue Arten der Gattung Hemicriconemoides *Chitwood and Birchfield 1957 (Nemata: Tylenchida)*

Drei neue Arten der Gattung *Hemicriconemoides,* nämlich *H. chitwoodi* n.sp., *H. minutus* n.sp. and *H. strictathecatus* n.sp. werden beschrieben. *H. chitwoodi* ähnelt am meisten *H. gaddi* (Loos, 1949) Chitwood & Birchfield 1957. Weibchen von *H. chitwoodi* haben einen längeren Mundstachel mit mehr abgerundeten Knöpfen als *H. gaddi*; Männchen von *H. chitwoodi* sind grösser und haben

eine Seitenlinie mehr. *H. minutus* ähnelt sehr stark *H. brachyurus* (Loos, 1949) Chitwood und Birchfield, 1957. Die Weibchen haben einen längeren Mundstachel und weniger Ringe auf der äusseren Haut als *H. brachyurus*. *H. strictathecatus* unterscheidet sich von allen beschriebenen *Hemicriconemoides*-Arten durch den Besitz eines einfachen tischähnlichen Kopfringels.

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Received for publication: December 30, 1959.

SHORT COMMUNICATION

L. J. Warner & N. G. Hague [1]), *An improved method of making perspex cavity blocks for the processing of Nematodes.*

Perspex cavity blocks made by the method described by Goodey (1957) have to be polished for a long time to remove scratches. By heating blocks of perspex in a compression mould this work has been eliminated.

The dimensions and construction of the mould from brass plate are shown in Fig. 1. (See next page). The convex dowel projections are 'sweated' into drilled and reamed holes in the base plate; they and the plate are highly polished. When the ends and sides have been attached by screws to the base the mould is ready for use.

½ hour to make.

A piece of ½ inch (1.25 cm) thick perspex 4.625" x 1.0" (11.5 x 2.5 cm) is placed in the

[1]) Imperial College Field Station, Sunninghill, Ascot, Berks., England.

mould in an oven at 118° C for 15 minutes, then the whole tightened in a vice until the projecting perspex surface is flush with the mould and left there until cold. Each cavity block takes about ½ hour to make.

GOODEY, J. B. (1957). *Laboratory methods for work with plant and soil nematodes.* Tech. Bull. 2, Minist. Agric., London, H.M.S.O. 3rd edition, 47 pp.

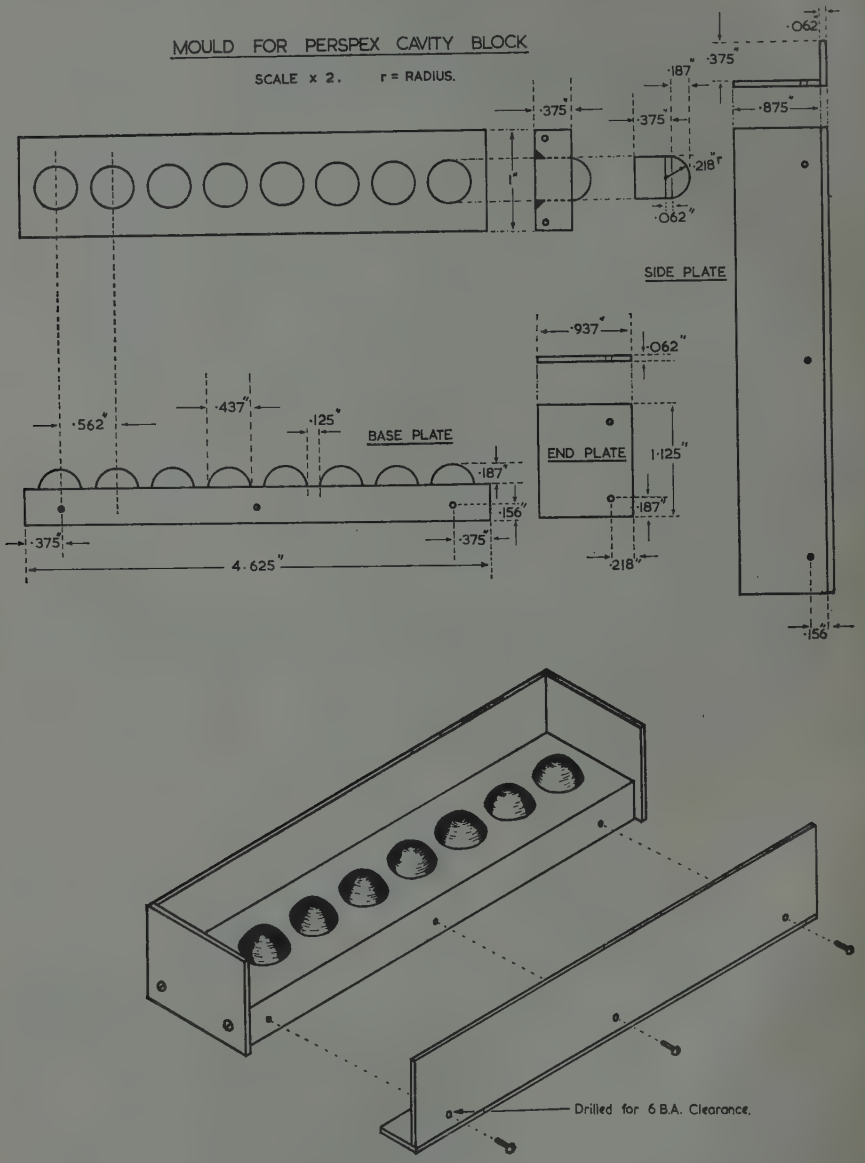

Fig. 1.

Nematologica 5 (1960): 73-77. E. J. Brill, Leiden

TELOTYLENCHUS, A NEW NEMATODE GENUS FROM NORTH INDIA (TYLENCHIDA: TELOTYLENCHINAE N. SUB-FAM.)

BY

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Nine females, four males and six larvae of a species of nematode rather like a *Tylenchorhynchus* were collected early in 1958 from soil around the roots of the grass, *Cynodon dactylon* Pers. at Aligarh, North India. The oesophageal glands lie in an elongated, cylindrical diverticulum overlapping the intestine for some distance, and resemble the situation in *Pseudhalenchus* (TARJAN, 1958). However, the new species has paired ovaries and the bursa envelops the male tail and so a new genus *Telotylenchus* is created for its reception.

A new subfamily Telotylenchinae is also erected to contain *Telotylenchus* and *Pseudhalenchus* because of their close similarity and their bad fit into the existing subfamilies of the Tylenchidae.

TELOTYLENCHINAE N. SUB-FAM.

Diagnosis. — Tylenchidae: Body elongate, cylindrical, transversely striated. Lateral fields marked by incisures. Head with six amalgamated lips, set off or continuous with body contour. Cephalic frame-work hexa-radiate, with little or no sclerotization. Buccal spear well-developed, with three basal knobs. Median oesophageal bulb well-developed, with distinct valvular apparatus. Isthmus not forming basal oesophageal bulb as in Tylenchinae but directly opening into intestine. Oesophago-intestinal junction indistinct. Oesophageal glands elongate, lying free in body cavity, extending back over anterior end of intestine. Nucleus of the dorsal oesophageal gland located posterior to sub-ventral gland nuclei. Ovary single or paired, outstretched. Vulva transverse. Testis single, outstretched. Tails of both sexes more than one anal body diameter long. Bursa present. Spicula and gubernaculum tylenchoid. Phasmids located on tail. *Type genus* — *Telotylenchus* n. g.

Telotylenchinae n. sub-fam. is closest to Tylenchinae from which it differs in the absence of the basal oesophageal bulb. It can not be justly accommodated in Hoplolaimidae because of the absence of the heavily sclerotized cephalic framework characterizing that family and also due to the difference in the structure and the position of the oesophageal glands and in the situation of their nuclei.

KEY TO THE GENERA OF TELOTYLENCHINAE

1. Ovaries paired, bursa enveloping entire tail . . . . . *Telotylenchus* n.g.
   Ovary single, bursa not enveloping entire tail . . . . . *Pseudhalenchus* Tarjan, 1958.

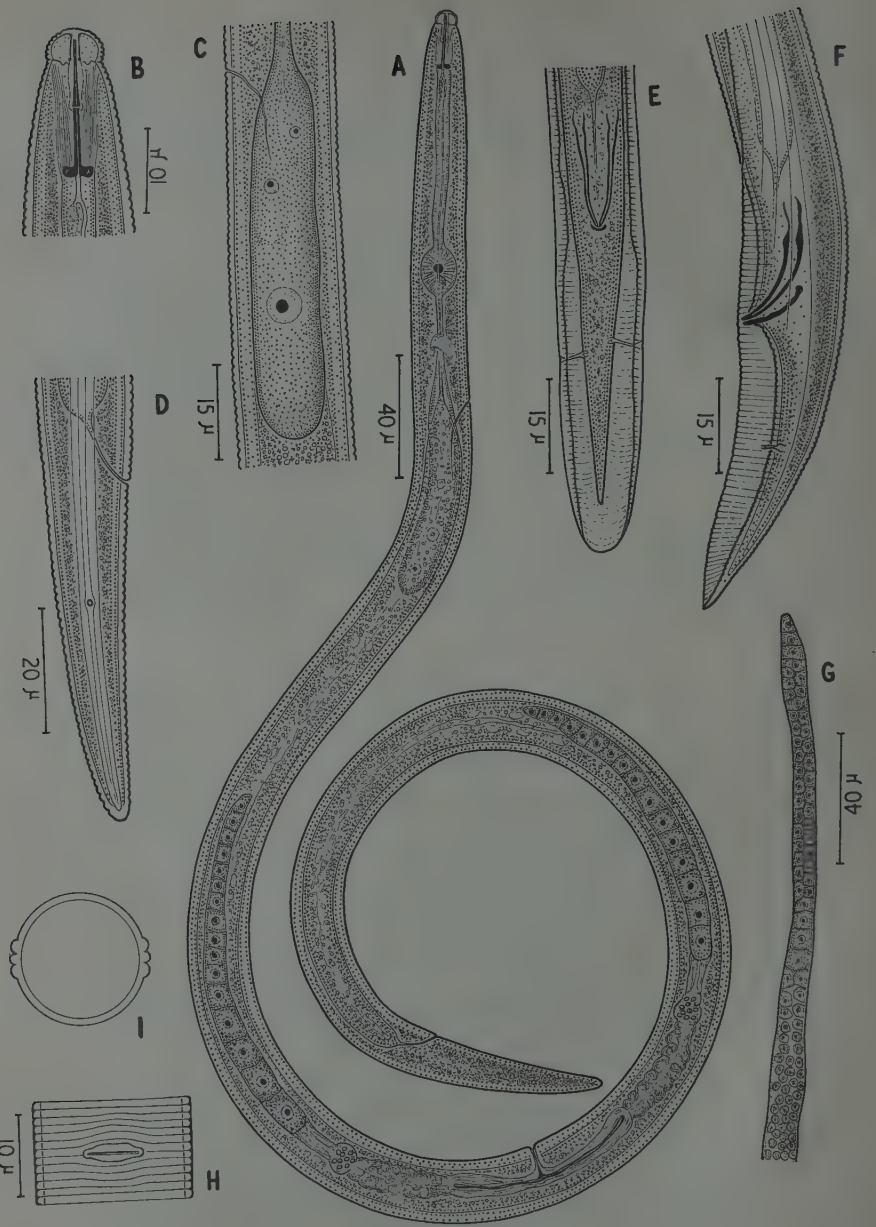

Fig. 1. *Telotylenchus indicus*: A. Female; B. Anterior end of female; C. Oesophageal glands of female in lateral view; D. Female tail in lateral view; E. Male tail in ventral view; F. Male tail in lateral view; G. Testis; H. Vulva in ventral view; I. Cross-section of female showing lateral fields.

Telotylenchus n.g.

Generic diagnosis. — Telotylenchinae: Both sexes similar in appearance. Body elongate, with distinct transverse striae. Lip region also with transverse striae. Labial frame-work not sclerotized. Buccal spear well-developed, elongate, with three basal knobs. Median oesophageal bulb with powerful valvular apparatus. Oesophageal glands elongate, extending back over anterior end of intestine; nucleus of dorsal gland large, located posterior to those of the sub-ventral glands. Oesophago-intestinal junction indistinct; cardia absent. Vulva situated near middle of body. Ovaries paired, outstretched, opposed. Female tail elongate-conoid, with blunt terminus. Male tail pointed, completely enveloped by bursa. Spicula and gubernaculum tylenchoid. Phasmids pore-like, located on tail. *Type species.* — *Telotylenchus indicus* n. g., n. sp.

Telotylenchus indicus n. g., n. sp.
(Fig. 1, A-I)

Measurements. — 9 ♀: L. = 0.7-0.88 mm; a = 35-43; b = 5.8-7.8; c = 14-17; V = 52-56; spear = 16-18 μ.

4 ♂: L. = 0.7-0.84 mm; a = 40-47; b = 6-7; c = 14-16; T = 50-63; spear = 15-16 μ; spicula = 22-24 μ; gubernaculum = 11-12.5 μ.

6 larvae: L. = 0.56-0.66 mm; a = 31-34; b = 5.4-5.9; c = 13-15; spear = 14-15 μ.

Female (Holotype). — L. = 0.84 mm; a = 42; b = 7.3; c = 16; V = 23-53-22.

Body elongate and slender, tapering regularly towards both extremities, assuming a slightly ventrally arcuate position when the eelworm is killed by gentle heat. Body cuticle with distinct transverse striae about 1 μ apart on middle of body. Lateral fields two-sevenths as wide as body, with four incisures of which outer ones are crenate. Deirids not observed. Head rounded, 7 μ wide, set off, button-like, from body by a deep constriction and marked by seven transverse striae. Labial frame-work hexa-radiate, not sclerotized, with inner margins forming a guiding tube for spear. Spear 17 μ long, with an anterior tapering tip and a posterior cylindrical shaft of equal length bearing three, strong, basal knobs measuring 4 μ across; outer margins of basal knobs slightly forwardly directed. Orifice of dorsal oesophageal gland located 3 μ behind spear base which is slightly concave. Precorpus a cylindrical tube, about 4 μ wide. Median oesophageal bulb slightly ovate, 14 μ long by 11 μ broad, highly muscular, with powerful, refractive, valvular apparatus in centre. Outlets of sub-ventral oesophageal glands located just behind valvular apparatus. Isthmus elongate, enveloped by nerve ring near its middle, connecting directly with intestine. Oesophageal glands cylindrical, three body-widths long, lying free in body cavity, extending back on left side of intestine. All the three glands almost amalgamated although a slight indication of their being separate is seen in some specimens; gland nuclei distinct, that of the dorsal gland larger than and located posteriorly to those of subventral glands (Fig. 1, C).

Excretory pore about one body-width posterior to level of nerve ring, near the indistinct oesophago-intestinal junction. Hemizonid obscure. Intestinal cells with small, refractive granules; lumen distinct throughout.

Vulva a transverse, depressed slit, 6.5 μ wide. Vagina at right angles to body axis, communicating with a muscular uterine chamber 62 μ long. Two sets of reproductive organs, outstretched in opposition, lying on right side of intestine. Uteri highly muscular, each ending distally in a spheroidal spermatheca which contains a few sperms. Oviducts short. Ovaries outstretched, each with about 16 oöcytes arranged in single file.

Rectum short, conspicuous, one anal body-diameter long; anus distinct. Tail elongate-conoid, 4 anal body diameters long, regularly tapering to a bluntly rounded, unstriated terminus. Phasmids dot-like, about one-third the distance down the tail.

Male (Allotype). — L. = 0.72 mm; a = 40; b = 6.2; c = 15; T = 50. Body similar to that of female. Lateral fields with four incisures, about three-elevenths of body-width broad. Lip region spherical, set off from neck by a deep constriction, bearing seven transverse striae. Buccal spear 16 μ long, similar to that of female in structure. Orifice of dorsal oesophageal gland 2.5 μ behind spear base. Median oesophageal bulb highly muscular, 13 μ long by 10 μ broad, with a refractive valvular apparatus. Oesophageal glands as in female.

Testis single, outstretched; spermatocytes arranged as illustrated (Fig. 1, G). Bursa large, distinctly crenate, arising at about one tail length anterior to cloaca, completely enveloping tail. Spicula paired, ventrally arcuate, cephalated, 23 μ long. Gubernaculum simple, appearing rod-shaped in lateral view, with proximal end knobbed and slightly bent ventrally, 11 μ long. Phasmids anterior to middle of tail, extending into bursa. Tail slightly, ventrally arcuate, regularly tapering to a pointed terminus. Incisures of the lateral field displaced on tail as illustrated (Fig. 1, F).

Larvae. — Body similar to that of female. Cuticle striations rather coarse, 1.4 μ apart on mid-body region. Lip region rounded, set off from neck contour by a sharp constriction, with seven to eight transverse striae. Buccal spear and oesophagus as described for types. Tail elongate-conoid, tapering to a smoothly rounded terminus.

Holotype. — Female collected on 13 January, 1958; slide no. PN/T/008 deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

Allotype. — Male; slide no. PN/T/009; other data same as for holotype.

Paratypes. — 8 females, 3 males and 6 larvae; other data same as for holotype. 1 female paratype with Dr. J. B. Goodey, Rothamsted Experimental Station, Harpenden, England.

Type habitat. — Collected from soil about roots of grass, *Cynodon dactylon* Pers.

Type locality. — Aligarh (U.P.), India.

DISCUSSION

TARJAN (1958) erected a new genus, *Pseudhalenchus* to accommodate species resembling those of the genera *Tylenchus* Bastian, 1865 and *Ditylenchus* Filipjev, 1934 but differing from them mainly in that the oesophageal glands considerably overlap the intestine. He placed this genus in Tylenchinae Filipjev, 1934. *Telotylenchus* n. g. is similar to *Pseudhalenchus* in many respects especially in the structure of the oesophagus. The Tylenchinae are characterized by the possession of a basal oesophageal bulb, so to contain the genera *Telotylenchus* and *Pseudhalenchus* a new subfamily Telotylenchinae is erected.

The position of the genus *Halenchus* Cobb, 1933 is still uncertain. COBB (1933) and CHITWOOD & CHITWOOD (1950) placed it in Tylenchinae while THORNE (1949) regarded it as a member of Nothotylenchinae of the family Neotylenchidae Thorne, 1949. In 1957, however, TARJAN emended the diagnosis of *Halenchus* placing it in Tylenchinae. As regards the structure of the basal portion of the oesophagus *Halenchus* should also belong to Telotylenchinae but, because the median oesophageal bulb and its valvular apparatus are indistinct in this genus, its inclusion in Telotylenchinae is avoided for the present.

The author is thankful to Dr. M. A. Basir under whose guidance this work was done.

ZUSAMMENFASSUNG

Telotylenchus, eine neue Nematodengattung aus Nordindien (Tylenchida: Telotylenchinae n. subfam.)

Telotylenchus indicus n. g. n. sp., der in Aligarh, Nord Indien, in der Nähe von Graswurzeln gefunden wurde, wird beschrieben und abgebildet. *Telotylenchus* gleicht am meisten *Tylenchorhynchus,* aber er unterscheidet sich durch die Abwesenheit eines hinteren Ösophagusbulbus und dadurch, dass die Ösophagusdrüsen frei in der Leibeshöhle liegen. *Telotylenchus* und *Pseudhalenchus* Tarjan, 1958 bilden zusammen eine neue Unterfamilie der Telotylenchinae.

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THE EFFECT OF SOME SINGLE ELEMENT DEFICIENCIES ON THE GROWTH OF *MELOIDOGYNE JAVANICA*

BY

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The effects on the development of parasites of elemental, nutritional deficiencies in the host have usually been studied on plant parasites because it is easy to develop single macronutrient element deficiencies in plants.

ALLEN & SELMAN (1956) found a significant drop in the rate of egg production in the mustard beetle *Phaedon cochleariae* when fed on watercress leaves deficient in N, P, K or Fe.

Similar effects have not been studied in nematodes but OTEIFA (1951) found that egg mass production by *M. incognita* considerably increased when the level of K changed from low to medium but that there was little further effect with further rise in K in the plant. OTEIFA (1953) found that *M. incognita* developed most slowly on lima beans with low K and fastest on those with high K. He reported (1952) correlation between increase in number of *M. incognita* on the plant and reduction of plant growth; infected plants had less N, P, K, Ca and Mg than controls. However, MAUNG & JENKINS (1959) found an increase in levels of N, P and K in roots of infected tomatoes with a heavy infestation of *M. incognita acrita,* but no significant changes with light infection.

Unhealthiness of tomatoes was not caused by the taking of 10% of the plant's total K by heavy infection of *Heterodera rostochiensis, M. incognita acrita* or *M. arenaria* (DROPKIN & KING, 1956).

This work was done to study the effect of complete, single element deficiencies in a host on growth and development of a parasite nematode *Meloidogyne javanica* (Treub) at levels of infection low enough to prevent host injury and competition between parasites.

MATERIALS AND METHODS

All the seeds used in each experiment came from a single susceptible tomato plant of the Pan America variety. They were sterilized in 5% sodium hypochlorite for 20 minutes and then washed six times in distilled water. These seeds were germinated on moist filter paper in large Petri dishes in the dark at 25° C and were then transferred on to a waxed mesh over 1/5 nutrient solution for several

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days. During this time they were aerated by bubbling air through the nutrient and care was taken to see that they were not exposed to extremes of temperature.

The nutrient solution was made from analytical reagents and glass-distilled water and was as follows: —

Macronutrients after ARNON & HOAGLAND, 1940

KNO_3	10	millemoles/litre
$NH_4H_2PO_4$	2	,, ,,
$Ca(NO_3)_2$	3	,, ,,
$MgSO_4.7H_2O$	2	,, ,,

Micronutrients after BROWNELL & WOOD, 1957

Fe	90	micromoles/litre
Bo	46	,, ,,
Mn	9.1	,, ,,
Zn	0.76	,, ,,
Cu	0.31	,, ,,
Mb	0.10	,, ,,

The Fe was supplied as ferric E.D.T.A. (JACOBSON, 1951). Table I shows how the deficient solutions were prepared.

TABLE I

Amounts of macronutrient elements (per litre of culture solution) added to various cultures in order to produce single element deficiencies.

	ml 0.5M $Ca(NO_3)_2$	ml 0.5M KNO_3	ml 0.5M $NH_4H_2PO_4$	ml 0.5M $MgSO_4.7H_2O$	
Full culture	6	20	4	4	
Less N	—	—	—	4	+ 0.75 g KCl, 0.28 g NaH_2PO_4, 0.27 g anhyd. $CaCl_2$
Less P	6	20	—	4	+ 0.11 g NH_4Cl
Less K	6	—	4	4	+ 0.85 g $NaNO_3$
Less Ca	—	20	4	4	+ 0.51 g $NaNO_3$
Less Mg	6	20	4	—	+ 0.64 g $Na_2SO_4.10H_2O$
Less S	6	20	4	—	+ 0.41 g $MgCl_2.6H_2O$
Less Fe	6	20	4	4	

Seedlings were held on four waxed corks fixed in holes in Perspex tops of polythene containers of 2 litre capacity. Air was kept bubbling through the nutrient solution. The containers were wrapped in "bible" paper with the black surface innermost to prevent algal growth and the white surface outermost to minimize heat absorption.

The seedlings were grown in a glass house, where the temperature fluctuated evenly between 7-36° C (45°-97° F) in the first experiment and 11-45° C (52°-113° F) in the second.

When the plants started to show deficiency symptoms and had suitable root growth, the "Perspex" lids were removed and were placed over similar containers

which had been cut down to half size so that the roots touched the bottom. Each of these containers had a similar number of freshly hatched *Meloidogyne javanica* larvae in 500 ml of 0.5% dialysed, oxygenated "Difco bacto-agar". The containers were left in the dark at 25° C and the plants were exposed to infection for 18 hours in the first experiment and for 24 hours in the glasshouse in the second, after which the roots were washed in distilled water and the infected plants were then returned to nutrients of various deficiencies and allowed to grow. Fresh nutrient solutions were made up every three weeks.

Plants were harvested at weekly or half weekly intervals, the galls fixed in 1% buffered osmium tetroxide for 2 hours at 8° C, washed and the nematodes dissected out. Their cross sectional area was measured in square microns (Bird 1959) but instead of using a camera lucida the image was projected on to the drawing paper from a constant distance. Dry weights of all plants were obtained at each harvest.

RESULTS

A pilot experiment showed that *M. javanica* grows very well on tomatoes in water cultures and, rather surprisingly, that it grows almost as rapidly on P deficient plants as on plants on full nutrient.

The first experiment lasted fifty-eight days and involved six harvests (Tables II and III). Deficiencies of each of the macronutrients and iron were employed

Table II

Average dry weights (grams) of infected tomato plants grown on various nutrients

Harvests	Full	Less N	Less P	Less K	Less Mg	Less S	Less Fe
1st (7 Days)	0.178	0.014	0.029	0.119	0.052	0.175	0.200
2nd (13 Days)	0.350	0.016	0.026	0.252	0.061	0.286	0.354
3rd (20 Days)	1.149	0.076	0.037	0.483	0.105	0.801	0.542
4th (30 Days)	2.900	0.032	0.036	0.960	0.078	2.750	0.555
5th (40 Days)	6.639	0.035	0.034	0.951	0.075	3.459	1.003
6th (58 Days)	12.626	0.096	0.033	1.460	0.102	4.283	1.110

and characteristic deficiency symptoms appeared in all plants except the controls. The plants grown in Ca deficient media were discarded because of the rapid breakdown of the root systems.

Neither P nor Mg deficient plants grew well, particularly towards the end of the experiment. Both, however, were capable of supporting nematodes which developed to their full size and, in the case of P deficiencies, were probably capable of laying viable eggs although this was not tested because the roots of these plants were starting to rot at this late stage. However, nematodes grown on Mg deficient plants laid viable eggs whose hatched larvae infected tomato seedlings.

TABLE III

Average cross sectional areas (1000 sq. μ) of nematodes grown in tomatoes on various nutrients

Age in days	Full	Less N	Less P	Less K	Less Mg	Less S	Less Fe
7	5.9(6)	7.2(4)	6.3(2)	6.3(7)	6.8(3)	5.7(9)	6.7(21)
13	12.4(13)	18.7(3)	11.3(3)	13 (4)	—	13.9(14)	15.1(2)
20	25.7(4)	28.3(3)	27.3(3)	24.8(5)	29.3(1)	18.9(6)	22.4(13)
30	30.5(6)	32.6(4)	30.5(3)	27.6(3)	39.4(1)	29.7(3)	31.1(3)
40	51.4(3)	139.6(3)	—	135.6(1)	—	83 (6)	90.1(7)
58	152 (1)	216 (5)	79 (2)	184 (4)	—	157 (9)	211 (3)

Number of nematodes in brackets.

The nematodes grew consistently faster in some of the deficient plants (Table III), notably those with deficiencies of N, Mg, Fe or K. The increase in growth rate of the nematodes occurred in Fe deficient plants when the deficiency showed

TABLE IV

Average dry weights of infected tomato plants grown on full nutrient and without N

Harvest	Control	Less N
1st 6 Days	0.022 g	0.007 g
2nd 14 Days	0.117 g	0.012 g
3rd 20 Days	0.274 g	0.009 g
4th 24 Days	0.658 g	0.013 g
5th 28 Days	1.107 g	0.011 g
6th 35 Days	4.527 g	0.016 g

TABLE V

Average cross sectional area of nematodes grown in tomatoes on full nutrient and without N and the percentage differences of the N deficient relative to full nutrient or control

Days	6	14	20	24	28
(C) Average cross sectional area (1000 sq. μ) and number of nematodes in brackets.	7.5 (5)	26 (9)	40 (5)	76 (12)	80 (13)
(N) Average cross sectional area (1000 sq. μ) and number of nematodes in brackets.	7.7 (3)	31 (7)	48 (5)	97 (15)	125 (9)
Differences relative to control (C) %	3	19	20	28	56

in the plants while in K deficient plants it occurred in the later stages only. Because of the low numbers of specimens in some cases (notably the Mg deficient plants) these results should be interpreted with caution.

Because of the unexpectedness of these results and of the possible effect of higher temperatures and more rapid growth, the experiment was repeated using plants on full nutrient and deficient in N. This second experiment lasted thirty-five days and involved six harvests (Tables IV and V). The last of these was only for collection of egg sacs. Growth of *M. javanica* was again more rapid in plants deficient in N than in healthy plants on full nutrient at low levels of infection (Fig. 1).

Fig. 1. Showing 24 day old infected tomato plants in full nutrient (full) and with an N deficiency (N). These plants and their parasites constitute the 24 day part of the second experiment.

Statistical analysis was carried out on the logarithm of the measurements. Variability on this scale appeared to be relatively uniform. The combined variences of all the data gave a standard deviation (on the log scale) of 0.10 which corresponds to a coefficient of variation of 23% in the untransformed data.

The differences between rates of growth of the parasites under these two treatments were highly significant ($P < 0.001$). The difference in relative growth rate (Fig. 2) was slight from 0-6 days and during moulting and greatest from 6-14 days and during the post moult phase. It appeared to be associated, as would be expected, with periods of maximum growth and feeding.

At twenty days 80% of the nematodes in the N deficient plants had completed moulting and 20% were still moulting, whilst only 20% of the nematodes in plants on full nutrient had completed moulting and 80% were still moulting. On the twenty-fourth day, 53% of the developing females in the N deficient plants had started to secrete egg sacs, compared with 8% of those in plants on full nutrient. In the first experiment also the nematodes in N deficient plants were

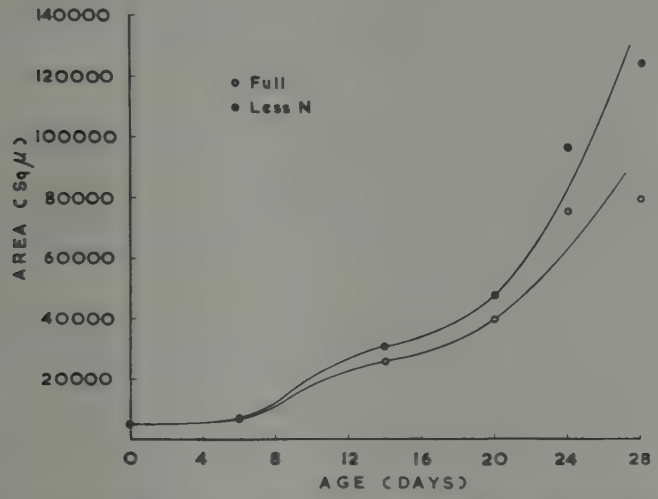

Fig. 2. Showing growth curves for nematodes grown on full nutrient (full) and with an N deficiency (less N).

the first to secrete egg sacs. About a third more larvae emerged from the egg sacs (collected at thirty-five days) from nematodes grown on N deficient plants than from those grown on plants on full nutrient. These larvae were viable and were observed to penetrate and develop in tomato seedlings.

DISCUSSION

Meloidogyne javanica parasitising tomato plants deficient in a single major element, responds in most cases, by growing more quickly than when it feeds on plants obtaining full nutrient. This response was most noticeable with N lack, and was present to a lesser degree in the absence of Mg, Fe or K.

Much work has been done on the effect of single element deficiencies on the metabolism of green plants. In the higher plants most of this type of work has been restricted to the leaves. Different genera and species often respond differently to single element deficiencies and, within the same species, there is a difference in amino-acid composition and total soluble N in the leaves, stem and roots of

plants grown on full nutrient (Steward, Crane, Millar, Zacharius, Rabson & Margolis, 1959). Under conditions of single element deficiency the amino-acid composition and total soluble N of the leaves show a different pattern for each deficiency.

Several explanations can be put forward for the changes in growth rate of *M. javanica* in response to single element deficiencies.

(1) The normal plant may partially resist the development of the nematode, reducing its growth rate slightly compared with stunted, deficient plants. This hypothesis seems unlikely, however.

(2) As a result of metabolic disturbance in the host the parasite may,
   (a) respond by growth acceleration
   or
   (b) find the products of this metabolic disturbance more suitable for growth than those associated with normal metabolism of the plant on full nutrient.

Tyler (1933) noticed that the percentage of males in cultures of *Meloidogyne* in tomato increased with increasing adverse conditions of nutrition and she suggested "that conditions of nutrition may be important enough in the early stages of development to change the physiological balance of certain individuals".

It is a common field observation that males appear in greatest numbers towards the end of the summer when plants are beginning to die and their roots are heavily infected. This is corroborated by Triantaphyllou & Hirschmann (1959) who found "that under conditions of food stress true males of *M. javanica* are abundant". In the first series of experiments described above males were found only on the N deficient plants. It is suggested that a similar type of stress causes the females to grow and produce eggs more rapidly. Under field conditions this type of response would have obvious survival value.

The observed increase in growth under conditions of single element deficiencies seems more likely to be associated with changes in the plant's metabolism, leading to accumulation of products beneficial to nematode growth.

To test these hypotheses, further work must be done on the metabolism of uninfected host roots and compared with similar studies on the metabolism of infected roots, in both cases using full nutrient and various single element deficiencies.

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ZUSAMMENFASSUNG

Der Einfluss des Mangels an einigen einzelnen Elementen auf dem Wachstum von M. javanica

Eine leichte Infektion durch *Meloidogyne javanica* bei Tomaten kommt einem Elementmangel bei der Wirtspflanze gleich. Die Nematodenentwicklung verläuft schneller beim Fehlen von N oder in geringerem Masse beim Fehlen von Mg, Fe oder K als wenn die Wirtspflanzen ihre vollständige Nahrungsmenge erhalten haben. Bei N-Mangel in der Wirtspflanze sind die Unterschiede im relativen Wachstumsmass signifikant. Sie sind am grössten während der Zeit des maximalen Wachstums und der Nahrungsaufnahme der Nematoden. Häutung und Eiersackproduktion treten schneller auf bei Nematoden in N-Mangelpflanzen als bei solchen, die bei voller Ernährung wachsen. Lebensfähige Eier und Larven werden in beiden Fällen produziert. Einige Erklärungen für diese unerwarteten Ergebnisse werden diskutiert.

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DIE NEMATODENFAUNA DES FICHTENROHHUMUS UNTER DEM EINFLUSS DER KALKDÜNGUNG

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Die Waldstreuzersetzung als mechanisch-biochemischer Prozeß ist für den Nährstoffkreislauf des Waldes von großer Bedeutung. Infolge Einwirkung verschiedener Faktoren kommt es durch Veränderung der Bodenreaktion zu einer tiefgreifenden Umschichtung der Organismengemeinschaft des Bodens und damit zu einer Anhäufung unzersetzten Bestandesabfalls. Mit zunehmender Versauerung wird durch Abnahme der Bodenbakterien die Streuzersetzung stark gehemmt und durch die daraus resultierende Rohhumusbildung dem Nährstoffkreislauf eine beträchtliche Menge an Mineralstoffen entzogen. Als billiges Mittel zur Neutralisation der Bodensäure hat sich die Kalkung erwiesen. Man strebt damit die biologische Aktivierung der Zersetzung an.

Aus bisher vorliegenden Untersuchungen von FRANZ (1959) ergibt sich, daß die Bodennematoden unter dem Einfluß der Kalkung eine deutliche Zunahme der Besatzdichte erfahren.

Da quantitative Befunde allein ungenügend sind, bemühten wir uns, das Verhalten der Nematodenfauna auch qualitativ zu erfassen, um einen Einblick in evtl. Umschichtungen des Artengefüges zu erhalten.

Es gelangten 2 Flächen zur Untersuchung:

I. Ein etwa 40-jähriger Fichtenstandort in Thüringen. Der Boden bestand aus festen Lehmschichten des oberen Buntsandsteins. Durch das kühle und regnerische Klima waren gute Voraussetzungen zur Rohhumusbildung geboten.
II. Etwa 30-jähriger Fichtenbestand auf Sandboden in Brandenburg, der ebenfalls zur Rohhumusbildung neigt.

Fläche I wurde 1952 mit 10.00 kg/hectare Branntkalk (CaO) und Fläche II 1958 mit 10.000 kg/hectare kohlensaurem Kalk ($CaCO_3$) gedüngt, indem der Kalk in einer dünnen Schicht ausgebracht wurde.

METHODE

Aus der Streuschicht und dem Rohhumushorizont wurden etwa im Abstand von 6 Wochen von Kalk- und Kontrollflächen je 10 Proben entnommen. Von jeder Einzelprobe wurden 4 ccm 10 Stunden im Baermann-Trichter aufgeschwemmt und danach quantitativ und qualitativ ausgewertet.

ERGEBNISSE

Wegen der großen Streuung der Einzelwerte war es erforderlich eine statistische Sicherung der Differenzen mit einer Grenzwahrscheinlichkeit von 5 % vorzunehmen (Tabelle I). Infolge der heterogenen Verteilung der Nematoden im Sub-

TABELLE I

Besatzdichte. Vergleich der Mittelwerte aus 10 Einzelproben bezogen auf 1 ccm

	Kalkfläche	Kontrolle	Sicherung (5%)
Hermsdorf			
13.3.1958	75	84	—
21.4.	115	65	—
27.5	133	90	—
28.7.	253	364	—
21.8.	160	369	—
21.11.	166	63	+
24.4.1959	139	105	—
7.11.	92	25	—
Tiefensee			
16.3.1959	76	60	+
15.5.	270	156	—
13.10.	89	75	—

strat erscheint es unzweckmäßig eine Umrechnung der Besatzdichte auf 1 qm vorzunehmen, wie das beispielsweise bei FRANZ der Fall ist. Die auftretenden Unterschiede erscheinen dadurch zwar viel krasser, bieten aber keine hinreichende Sicherheit. Bei unseren Untersuchungen zeigte sich in den meisten Fällen auf den Kalkflächen eine Zunahme der Nematodenpopulation von durchschnittlich 25 bis über 100 %. Es war jedoch nur in 2 Fällen möglich, diese statistisch gesichert auf Kalkungseinfluß zurückzuführen. Es zeigt sich also, daß selbst derartige offensichtliche Unterschiede, wie beispielsweise am 7.11.1959 von 92 : 25 Nematoden nicht gesichert werden konnten und deshalb wohl noch innerhalb der normalen ökologischen Streuungsbreite liegen dürften. Besondere Zurückhaltung ist deshalb bei Rückschlüssen auf Großflächenbesiedlung geboten.

Für die qualitative Analyse wurden folgende, auf den Untersuchungsflächen gefundene und bestimmbare Arten herangezogen (Tabelle II).

Verschiedene Arten bevorzugen die Kalkungsfläche. Die Mehrzahl konnte auf beiden Flächen nachgewissen werden. Die Individuenzahl der Arten, die nur auf den gekalkten Flächen auftraten, war aber meist so gering, daß sie nur als Zufallsfunde aufgefaßt werden und zu deren Charakterisierung nicht herangezogen werden können.

Um evtl. Schwankungen des Artengefüges trotzdem zu erfassen, wurde versucht, die absolute Individuenabundanz der Tiere, die mindestens mit einer Dominanz von 1 % auftraten, vergleichend darzustellen. Es handelt sich um Mittelwerte aus 10 Einzelproben, die auf 1 ccm bezogen wurden.

Tabelle II

Auf den Untersuchungsflächen gefundene und bestimmbare Nematoden-Arten

	Hermsdorf	Tiefensee
Rhabditis-Larven	×	×
Bunonema ditlevseni Micoletzky	+	
B. penardi Stefansky	×	
B. richtersi Jägerskj.		×
B. reticulatum Richters		+
Panagrolaimus-Larven	×	×
Cephalobus nanus De Man	×	×
Eucephalobus striatus (Bastian)	+	
E. oxyuroides (De Man)		×
E. elongatus (De Man)	+	×
Acrobeloides bütschlii (De Man)	×	×
Cervidellus vexilliger (De Man)		×
Teratocephalus crassidens De Man	×	×
T. terrestris (Bütschli)	×	×
Tylenchus davainii Bastian	×	
T. filiformis Bütschli	×	×
T. thornei Andrassy	×	+
T. agricola De Man	×	×
T. leptosoma De Man	+	×
T. bryophilus Steiner	×	×
T. sp. I	×	×
Ditylenchus intermedius (De Man)	×	×
Ecphyadophora tenuissima De Man	×	
Aphelenchoides parietinus (Bastian)	×	×
Plectus cirratus Bastian	×	
P. rhizophilus De Man	×	×
P. longicaudatus Bütschli	×	×
P. communis Bütschli		+
P. geophilus De Man	+	+
P. parvus Bastian	×	×
Wilsonema otophorum (De Man)	×	×
Monhystera dispar Bastian		+
M. similis Bütschli	+	+
M. vulgaris De Man	×	×
M. filiformis Bastian	×	+
Prismatolaimus dolichurus De Man	×	×
Mononchus papillatus (Bastian)		+
Prionchulus muscorum (Dujardin)		+
Dorylaimus parvus De Man	×	×
D. carteri Bastian	×	×
D. centrocercus De Man		×
D. obtusicaudatus Bastian	+	
Tylencholaimus minimus De Man	+	
T. stecki Steiner	+	
Triplonchium cylindricum Cobb	+	
Alaimus primitivus De Man	×	

× Vorkommen auf Kalk- und Kontrollfläche,

+ ausschließliches Vorkommen auf der Kalkungsfläche.

Tabelle III

Absolute Individuenabundanzen. + = *Kalkungsfläche,* — = *Kontrollfläche*

	Hermsdorf										Tiefensee					
	I		II		III		IV		V		VI		VII		VIII	
	28.7. 58		21.8. 58		21.11. 58		24.4. 59		7.11. 59		16.3. 59		15.5. 59		13.10. 59	
	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
habditis-Larver															1	
unonema richtersi											1	1				
unonema-Larven	3															
anagrolaimus-Larven											1		2			
ephalobus nanus	35	2	20	2	6	1	5	2	4		8	16	6	8	6	9
ucephalobus elongatus											1	2				
crobeloides bütschlii			7	1			3		1							1
ervidellus vexilliger													7	13	3	9
eratocephalus crassidens	3	6	2	5	2	7	4	6	1		9	4	17	5	1	1
. terrestris	1	1	1			1	4	6	1		10	6	8	11	2	2
ylenchus davainii	5	4	4	4	1											
. filiformis	9	24	10	23	30	2	5	4	5	2	2	4	8	10	1	2
. thornei	5	9	1	3	1											
. agricola		2					3		6		1	1	7	5		1
. leptosoma	3															
. bryophilus							18	2	2				1	4		6
. sp. I	10	5	6	5	3	2			3	13	4	6		5	4	4
ylenchus-Larven	11	41	15		26	10	37	6	8	4	6	2	18	6	2	8
itylenchus intermedius					1	1	1	2	1		1	2	1		2	2
cphyadophora tenuissima				1												
phelenchoides parietinus	48	45	17	20	48	21	3	27	14	3	12	4	48	43	19	17
lectus longicaudatus	2			1	1	1					3		1			1
. geophilus					1		4									
. parvus				3		1	1				2	4	2		11	
Vilsonema otophorum											14	5	16	23	17	13
Monhystera similis	3	1											1			
M. vulgaris	2		3						44							
M. filiformis			9	8	15	2							2			
M. dispar			2				24									
M. -Larven	14	5	5				3									
rismatolaimus dolichurus	2	7	1	1		2	24						14			
Mononchus papillatus											1		1		1	
Dorylaimus carteri	2	1	1	1	3		1		2		2	1	5		2	
Triplonchium cylindricum			2													

Auf den verschiedenen Flächen kommt es zu teilweise erheblichen Abundanzschwankungen (Tabelle III). Für *Cephalobus nanus* ergibt sich eine deutliche Zunahme auf den Kalkflächen, ebenso für die verschiedenen *Tylenchus*- und *Monhystera*-Arten. Die starken Schwankungen von *Aphelenchoides* lassen keine eindeutigen Schlußfolgerungen zu. Für die Fläche Tiefensee ergeben sich keine

klaren Unterschiede. Die Entwicklung der Gattung *Tylenchus* aber ist ebenfalls auf der Kalkungsfläche bedeutend stärker. Im Gegensatz zu Hermsdorf tritt hier *Wilsonema otophorum* viel häufiger auf. Zu ganz exakten Aussagen wäre es angebracht, die Besatzdichteunterschiede der einzelnen Arten ebenfalls statistisch zu erfassen.

Der durch die Kalkung erzielte pH-Anstieg von 3 auf 5 dürfte nicht unmittelbar auf die Nematoden wirksam werden, da sie in dieser Beziehung recht widerstandsfähig sind, sondern mittelbar über Änderung der Ernährungsverhältnisse. Die Erhöhung der Nematodenzahl auf den Kalkungsflächen ist sicher auf die dort eingetretene Zunahme an Bakterien zurückzuführen, wodurch sich die Ernährungsbedingungen der Bakterienfresser wesentlich gebessert haben. Für die stacheltragenden Nematoden müßte man eigentlich durch die Abnahme der Pilzhyphen, die als Nahrungsquelle angestochen werden, mit einer Reduktion rechnen. Durch die günstigen Lebensbedingungen jedoch hat sich wohl die Masse der Feinwurzeln als Nahrungsquelle beträchtlich erhöht, so daß die Zunahme vielleicht dadurch und die größeren Schwankungen durch die Umstellung der Ernährung zu erklären sind. Vielleicht ist das konstante, wenn auch geringe Auftreten von *Mononchus* auf den Kalkflächen darauf zurückzuführen, daß auf diesen die Beutetiere eine stärkere Besiedlungsdichte aufweisen.

Eine Gesamtbetrachtung des Artenspektrums zeigt, daß im Fichtenrohhumus die echten Parasiten und die Saprozoen stark zurücktreten oder völlig fehlen. Es überwiegen Vertreter der Gattungen *Cephalobus, Tylenchus, Aphelenchoides, Plectus* und *Monhystera.* Bei den in der Gesamtübersicht aufgeführten *Rhabditis*-Larven dürfte es sich wohl im wesentlichen um *R. aspera* Bütschli und *R. filiformis* Bütschli handeln, wobei letztere durch die lange und schmale Mundhöhle charakterisiert wird. Der Schwanz war etwas kürzer, als in der Literatur angegeben. Interessant ist weiterhin der für Deutschland erstmals erfolgte Nachweis von *Ecphyadophora tenuissima* in etwa 30 Exemplaren. Die Bestimmung war durch die typische Bursa-Ausbildung der Männchen einwandfrei möglich. Die von uns gefundenen Tiere entsprechen der ausführlichen Beschreibung von Tarjan (1957) völlig. Die Ausbildung eines Mittelbulbus war ebenfalls nicht feststellbar, so daß die Einordnung zu den Neotylenchinen völlig gerechtfertigt erscheint.

Die unter *Tylenchus* sp. I aufgeführte Art war trotz ihres häufigen Vorkommens nicht sicher zu bestimmen. Die Größe betrug etwa 0,4 mm, der kleine und schwache Stachel war aber gut sichtbar geknöpft. Bursa schwach ausgeprägt. Nach all diesen Merkmalen würde ich die Art als *Tylenchus discrepans* Andrassy ansehen. Nur der Schwanz ist ziemlich kurz und nicht spitz auslaufend.

An der Identität von *Triplonchium cylindricum* besteht nach Ausbildung des Stachels und des Kopfes kein Zweifel, obwohl nur eine Größe von 0,8 mm erreicht wird.

Allgemein zu der Kalkung wäre noch zu sagen, daß mit einer langjährigen Wirkung zu rechnen ist, da die ausgebrachten Mengen nur langsam verbraucht

werden. Irgendeine Schädigung war nicht nachzuweisen, weder im quantitativen noch qualitativen Nematodenbesatz. Durch Konzentrationsunterschiede und verschiedene Kalksorten zeigten sich keine Unterschiede in der Wirksamkeit.

Den Herren Dr. Paesler und Dr. Kühn, Naumburg danke ich für ihre Unterstützung. Herr Prof. Dr. Gäbler, Direktor des Instituts für Forstzoologie Eberswalde, förderte die Arbeit in großzügiger Weise.

SUMMARY

The influence of liming on the nematodes found in raw pine humus

The addition of $CaCO_3$ or CaO to raw pine humus increases its nematode population by from 25 to 100 per cent. The species present, however, remain essentially the same. Cephalobinae, Monhysterinae and Tylenchinae react most, sometimes by a considerable increase in population density. This may be the result of changed feeding conditions. Lime never caused decreases in population and various forms of lime and various dosages gave much the same result. *Ecphyadophora tenuissima* De Man was found for the first time in Germany.

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THE TAXONOMY OF *HYDROMERMIS CONTORTA* (LINSTOW, 1889) HAGMEIER, 1912, AND ITS SYNONYM *HYDROMERMIS RIVICOLA* CORTI, 1902, (NEMATODA: MERMITHIDAE) WITH NOTES ON SOME DIAGNOSTIC CHARACTERS

BY

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KOHN's (1905) emendation of *Mermis contorta* Linstow, 1889, initiated a controversy that probably will continue for a long time in mermithid literature. My contribution to this debate is based on specimens from the Lea River, Harpenden, Herts., England, type specimens from Germany, and an examination of the literature.

Mermis contorta was described from one immature male and female, and three mature females collected in a ditch at Göttingen, Germany. KOHN (1905) described material from *Chironomus plumosus* (L.) collected in Austria. His specimens differed from Linstow's in the number of longitudinal chords and the absence of cuticular fibres, but Kohn considered these were minor differences, or errors on Linstow's part and believed his and Linstow's material were the same species. He also transferred Linstow's species to *Paramermis* Linstow, 1898, and criticized Corti's erection of *Hydromermis* in 1902. LINSTOW (1905) and CORTI (1906) defended their observations, the latter proposing the name *Hydromermis implicata* Corti, 1906, for Kohn's form, a name that has had little acceptance. KOHN restated his views in 1906 and pointed out similarities between his own and Corti's material.

Later authors accepted Linstow's and Kohn's material as one or two species, depending largely on their acceptance or rejection of *Hydromermis* as a valid genus. DADAY (1911 and 1913) recognized the genus and thus, two species, *P. contorta* (Linstow, 1889) Kohn, 1905, and *H. contorta* (Kohn, 1905) Daday, 1911. STILES & HASSALL (1920) indicated two species, as did POLOZHENTSEV (1954) in his check list of mermithids. JOHNSON (1956) supported Daday's assignment of Kohn's *contorta* to *Hydromermis* in his recent study of the eelworm in United States. HAGMEIER (1912) considered the two forms synonymous as he used the designation *P. contorta* (Linst., 1889) emend. Kohn, 1905. Hagmeier's

designation was accepted by STEINER (1918 and 1919), SVABENIK (1928) and COMAS (1927 and 1928).

The main difference between the specimens of LINSTOW (1889, 1905) and those of KOHN (1905, 1906) and, indeed between the genera *Paramermis* and *Hydromermis* is the number of hypodermal chords: members of the first genus

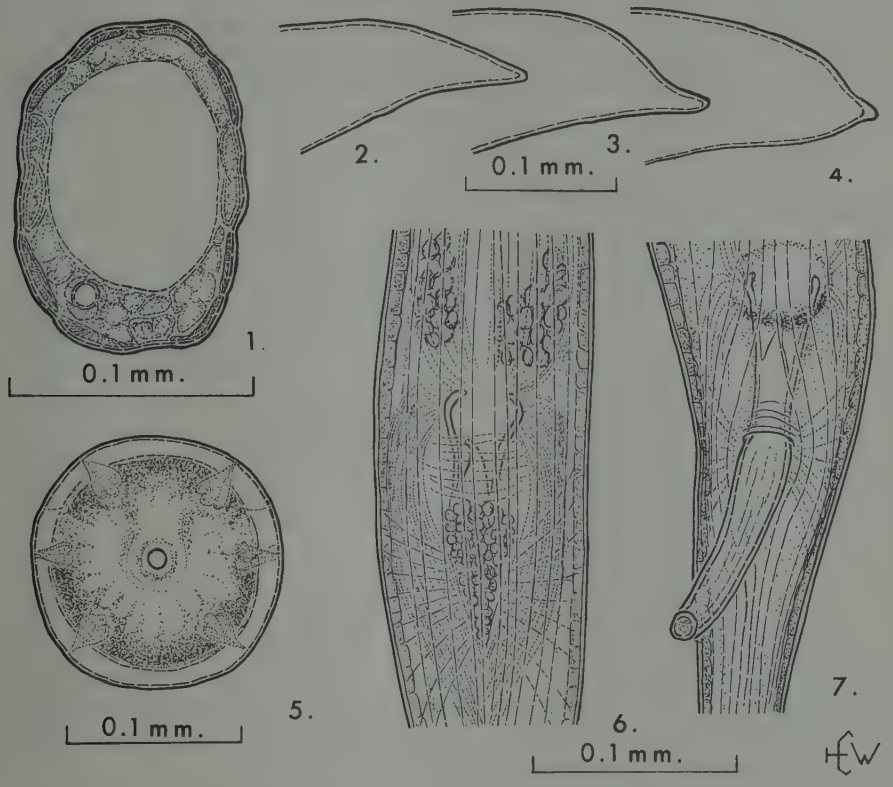

Fig. 1. Cross-section of a syntype of *Mermis contorta* Linstow, 1889. Fig. 2, Conoid; Fig. 3, Subdigitate; Fig. 4, Digitate termini of *Hydromermis contorta* (Linstow, 1889) Hagmeier, 1912, from Lea River, Harpenden, Herts., England. Fig. 5, En face view of *H. contorta*. Fig. 6, Dorsal view of spicule base of *H. contorta* showing paired muscle insertions. Fig. 7, Ventral view of spicule of *H. contorta*.

have six, the latter genus, eight. The number of hypodermal chords is difficult to determine and requires careful examination of cross-sections. STEINER'S (1929) suggestion that eight chords is the basic number is generally accepted. The subdorsal chords are usually considered missing, but on close examination appear as thin outpouchings of the hypodermis in the subdorsal region, or lie closely parallel to the dorsal chord.

Through the kindness of Dr. G. Hartwich, Zoologisches Museum, Humboldt-Universität, Berlin, Germany, I was able to examine cross-sections of one of two specimens (No. Q 3968) identified by Linstow as *Mermis contorta*. Eight hypodermal chords were present indicating that at least one of Linstow's specimens was of the genus *Hydromermis* (Fig. 1).

Another apparent difference in the species, involved the presence or absence of criss-cross fibres in the cuticle. Linstow did not describe fibres, though he illustrated a curious mechanical criss-cross pattern (1889, Plate 22, Fig. 1). Kohn interpreted this pattern as fibres, and noted that such fibres were absent in his own material. Corti (1906) cast doubt on this dissimilarity when he reported that Linstow stated *in litt.* that the cuticle of his specimens was simple, that is without fibres. Neither of the two specimens of Linstow that I examined had criss-cross fibres in the cuticle, so that Linstow's material must be the same as Kohn's in this regard. This and the previous observation concerning the chords leads to the conclusion that Linstow's specimens were of the genus *Hydromermis*.

Kohn (1905), Hagmeier (1912), and Johnson (1956) observed that the gonads of *Mermis contorta* were well developed at emergence and that the free-living eelworms did not moult; the final moult presumably occurs before emergence. In other aquatic mermithids, as those described by Strickland (1911), Iyengar (1928), Welch (1960) and numerous records from mosquitoes (Jenkins & West, 1954), the gonads are undeveloped upon emergence, and the eelworms moult and mature later. Maturity at emergence in *M. contorta* appears uncommon and therefore is noteworthy. Linstow (1889) gave no observations on the biology of his specimens, so that the host and age of the material are unknown. He claimed that one specimen had a larval appendage formed by the cuticle of the last larval stage. Whether the presence or absence of larval cuticle is significant is debatable, especially in view of its presence in only one of Linstow's original specimens. Kohn (1905, Plate 16, Fig. 3 and 4) illustrated mature eelworms which appear to have appendage-like termini. Approximately one-third of my material had the same appearance (Fig. 4). Without an illustration or detailed description it is difficult to decide whether Linstow's specimen had a true larval appendage, or an adult tail with an appendage-like terminus.

The evidence suggests that Linstow's, Kohn's, Hagmeier's and Johnson's material were the same species and of the genus *Hydromermis*. In an addendum to his publication Hagmeier (1912) suggested that if *Hydromermis* were accepted then *contorta* would be of that genus. This constitutes an indication and gives Hagmeier the authority for the combination.

A second aspect of this problem concerns *H. rivicola* Corti, 1902. This species was also recorded from a chironomid, *Chironomus venustus* Staeg., of the same subgenus as *C. plumosus*. Kohn (1905) separated his species from *rivicola* by the shape of the tail. Corti (1905), however, said that the shape of the tail in his specimens was the same as in Kohn's, but that there were differences between both the gonads and oesophagus. Kohn (1906) reviewed these differences and

concluded that they were minor. The closely related hosts, the maturity at emergence, the shape of the terminus, and spicule shape and size suggest that Kohn's material was synonymous with Corti's. As the two species appear synonymous, then *Hydromermis contorta* (Linstow, 1889) Hagmeier, 1912, becomes the type of the genus *Hydromermis*.

The specimens from the Lea River, Harpenden, Herts., England, comprising twenty-five females and four males, provided an opportunity to investigate some of the diagnostic characters.

The body proportions of the English material are comparable to those given by other authors for localities in Europe (Table I).

TABLE I

Ranges of measurements of the adult females of Hydromermis contorta *(Linstow, 1889) Hagmeier, 1912*

Author		Length	Width	Head Width	Host
Linstow (1889)	♂	14.8	0.17	—	unknown
Göttingen	♀	24.1-49	0.23 —0.28	—	
Corti (1902)	♂	15-32	0.18 —0.28	0.072 —0.097	*C. venustus*
Italy	♀	18-56	0.23 —0.45	0.050 —0.085	
Kohn(1905)	♂	13-26	0.07 —0.21	—	*C. plumosus*
Austria	♀	26-50	0.18 —0.37	—	
Hagmeier (1912)	♂	14-25	0.13 —0.19	0.053 —0.075	*C. plumosus*
Heidelberg	♀	19-40	0.18 —0.24	0.062 —0.075	
Welch	♂	11-16	0.12 —0.16	0.043 —0.069	*Chironomus* sp.
England	♀	15-41	0.17 —0.32	0.043 —0.064	
Average:	♀	26.2 ± 1.6	0.24 ±0.01	0.055 ±0.002	

All measurements in millimetres

KOHN (1905) illustrated four types of female termini among his material. Three of these, the conoid (Fig. 2), subdigitate (Fig. 3), and digitate (Fig. 4) occur in the English material in the following percentages: 41, 32, and 27. Though the females with conoid termini are shorter on the average than the subdigitate, and these in turn than the digitate females, the differences are not statistically significant. The shape of the terminus also appears unrelated to the extension of the trophosome into the tail region, or to the relative degree of gonad development. Terminal shape was also unrelated to papillae and amphid location as found by STEINER (1918) in *Hydromermis annulosa* Daday. The shape of the terminus appears unrelated to eelworm size or development, but is quite distinct for this species compared to the blunt shape of other species of the genus.

The nerve ring and associated ganglia of the English material is similar to that of Hagmeier's material. The forward extension of the trophosome often pushes the nerve structure forward and alters its relative position to the excretory pore.

In face view the six papillae groups are evenly spaced hexagonally about the mouth, (Fig. 5). The amphidial pores are slightly dorsal to the lateral papillae.

The spicular structure is of the same length, 0.24-0.27 mm, and width, 0.026-0.034 mm, as that found by other investigators. In lateral view it appears as a single structure (HAGMEIER, 1912, Plate 20, Fig. 44), but in dorsal or (Fig. 6) ventral view (Fig. 7) there are two spicules fused for most of their length. Their proximal ends are separate, while the distal portions are fused into a structure that is circular in cross-section (Fig. 7). In dorsal view the two heads of the spicules may be seen with the paired insertions of the retractor and protractor muscles (Fig. 6).

No evidence of intersex anomalies was found in any of the twenty-nine specimens.

ZUSAMMENFASSUNG

Die Taxonomie von Hydromermis contorta (*Linstow, 1889*) *Hagmeier, 1912, und ihres Synonym,* Hydromermis rivicola *Corti, 1902, (Nematoda: Mermithidae) mit Notizen über diagnostische Eigenschaften*

1. Die Untersuchung einer Syntype von *Mermis contorta* Linstow, 1889, zeigte a) keine gekreuzte Fasersicht in der Cuticula b) acht hypodermale Längslinien. Deshalb gehört diese Art in das Genus *Hydromermis* Corti, 1902, wie von HAGMEIER (1912) vorgeschlagen wurde. Sie muss dort in der Kombination *Hydromermis contorta* (Linstow, 1889) Hagmeier, 1912 erscheinen.

2. Die Art *Hydromermis rivicola* Corti, 1902 ist ein Synonym zu *H. contorta* (Linstow, 1889) Hagmeier 1912; deshalb hat *H. contorta* die Priorität und wird der Genotyp.

3. Bei Überprüfung von Tieren, die in England gesammelt wurden, konnten drei der vier weiblichen Termini, die von KOHN (1905) beschrieben wurden, aufgefunden werden. Das einzige Spiculum von *H. contorta* ist durch Fusion zweier Spicula entstanden.

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RHADINAPHELENCHUS COCOPHILUS (COBB, 1919) N. COMB., THE NEMATODE ASSOCIATED WITH "RED-RING" DISEASE OF COCONUT

BY

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Aphelenchoides cocophilus (Cobb, 1919) T. Goodey, 1933 has never been adequately described. LORDELLO & ZAMITH (1954) had fresh material and made some new observations on its morphology, but they overlooked two important characters, the terminal, bursal flap on the male tail, and the flap of cuticle overlying the vulva in the female. These and other characters separate the species from all others placed in *Aphelenchoides* by T. GOODEY (1951) and necessitate erecting a new genus *Rhadinaphelenchus* n.g. to accomodate it. Its slender nature and lithe appearance when swimming in water make the word ῥαδινός appropriate for the forepart of the generic name.

Three years after COBB (1919) first described the nematode, MICOLETZKY (1922) placed it in his subgenus *Chitinoaphelenchus,* but T. GOODEY (1933) transferred it to *Aphelenchoides.* CHITWOOD in CORBETT (1959) raised *Chitinoaphelenchus* to generic rank and replaced *A. cocophilus* in the genus. The type of *Chitinoaphelenchus* was designated as *Aphelenchus (C.) ormerodis* Ritzema Bos, 1891 (Micoletzky, 1922) by CHITWOOD (1935) who followed T. GOODEY (1928) in making it a synonym of *Aphelenchoides parietinus.* T. GOODEY (1951) maintained the status of *A. ormerodis* as a synonym of *Aphelenchoides parietinus* but ALLEN (1952) made part of the conception of *A. ormerodis* a synonym of *Aphelenchoides fragariae,* though he presented no arguments for this. FRANKLIN (1955) suggested that *A. ormerodis* was similar to *Aphelenchoides ritzema bosi.* In view of these divergent opinions and the confusing nature of the original description, I prefer to treat *A. ormerodis* as a *species inquirenda.* Whatever the status of *A. ormerodis* and of the subgenus or genus *Chitinoaphelenchus, A. cocophilus* is not closely related to *A. ormerodis* or to *Chitinoaphelenchus* and has to be placed in a separate genus.

The terminal bursal flap of the male suggests an affinity with *Bursaphelenchus* and the vulval flap of the female one with *Laimaphelenchus,* but the species does not fit comfortably into either of these genera.

The following new description is based on living specimens from Trinidad contained in fresh pieces of young coconut stem suffering from "Red-ring" disease.

Rhadinaphelenchus n.gen.

Aphelenchoididae. Very slender nematodes (a ≏ 100); female with vulval flap, long post-vulval uterine sac, long digitate tail; male tail with terminal, bursal flap. Type species: *Aphelenchus cocophilus* Cobb, 1919.

Rhadinaphelenchus cocophilus (Cobb, 1919) n. comb.

syn. *Aphelenchus cocophilus* Cobb, 1919

A. (Chitinoaphelenchus) cocophilus Cobb, 1919 (Micoletzky, 1922)

Aphelenchoides cocophilus (Cobb, 1919) T. Goodey, 1933

Chitinoaphelenchus cocophilus (Cobb, 1919) Chitwood in Corbett, 1959

Measurements were made on specimens mounted in glycerine after fixation in TAF (n = 10) but for b (n = 2).

♀ L = 1.05 mm (0.97-1.18); a = 87 (78-96); b = 8.7; c = 11.6 (9.5-13.2); V = $^{36}66^{20}$ ($^{25\text{-}41}64\text{-}68^{18\text{-}21}$)

♂ L = 1.02 mm (0.84-1.16); a = 120 (100-179); b = 6.5; c = 28 (24-35); T = 59 (50-68)

After Cobb (1919) n =?

♀ L = 1 mm; a = 71; b = 7.7; c = 12.5; V = $^{28}68^{13}$

♂ L = 1 mm; a = 100; b = 7.7; c = 20; T = 45

After Lordello & Zamith (1954) n = 5

♀ L = 0.83-1.11 mm; a = 60-96; b = 11-14; c = 9.4-10.7; V = 66-69; spear = 15.3 μ

♂ L = 0.82-1.42 mm; a = 92-143; b = 11-19; c = 22-47; spear = 10.7-13.8 μ

Very slender nematodes about 1 mm long, tapering very slightly anteriorly but clearly and gradually from the vulva posteriorly; female measures only 13.5 μ at greatest body-width, male 10.5 μ. Body with very faint annules, visible on live specimens, but on fixed and mounted ones visible only on anterior part of body, where annules are about 0.5 μ wide. Head offset, narrower than body, about 5.5 μ wide and just over 3 μ high, not annulated. Lateral fields with four incisures and a faint line suggesting the presence of a middle fifth incisure; outer incisures crenate. Lateral fields begin just anterior to median oesophageal bulb and end just beyond anus. Each field about a quarter body-width wide for most of its length but narrowing at each end. Phasmids and deirids not seen. Spear 12-13 μ long in both sexes, anterior part very fine, about 5 μ long, a median part somewhat wider but only about 0.4 μ wide, and a basal part about 3 μ long and, although wider still, only about 1 μ wide (Fig. B). Head framework consists of a minute, hexaradiate, ribbed cylinder through which the spear tip moves (Fig. D). On the sides of the head the ribs of this thickening appear as fine bars which bifurcate basally (Fig. C); Cobb's (1919) drawing somewhat exaggerates their delicacy. Of the oesophagus only the median bulb is clearly visible, as an oblong structure, with rounded corners, about twice as long as broad with the crescentic valve plates just behind its middle (the slight constriction mentioned by Lordello & Zamith (1954) was not observed, but this kind of constriction can be observed as a fixa-

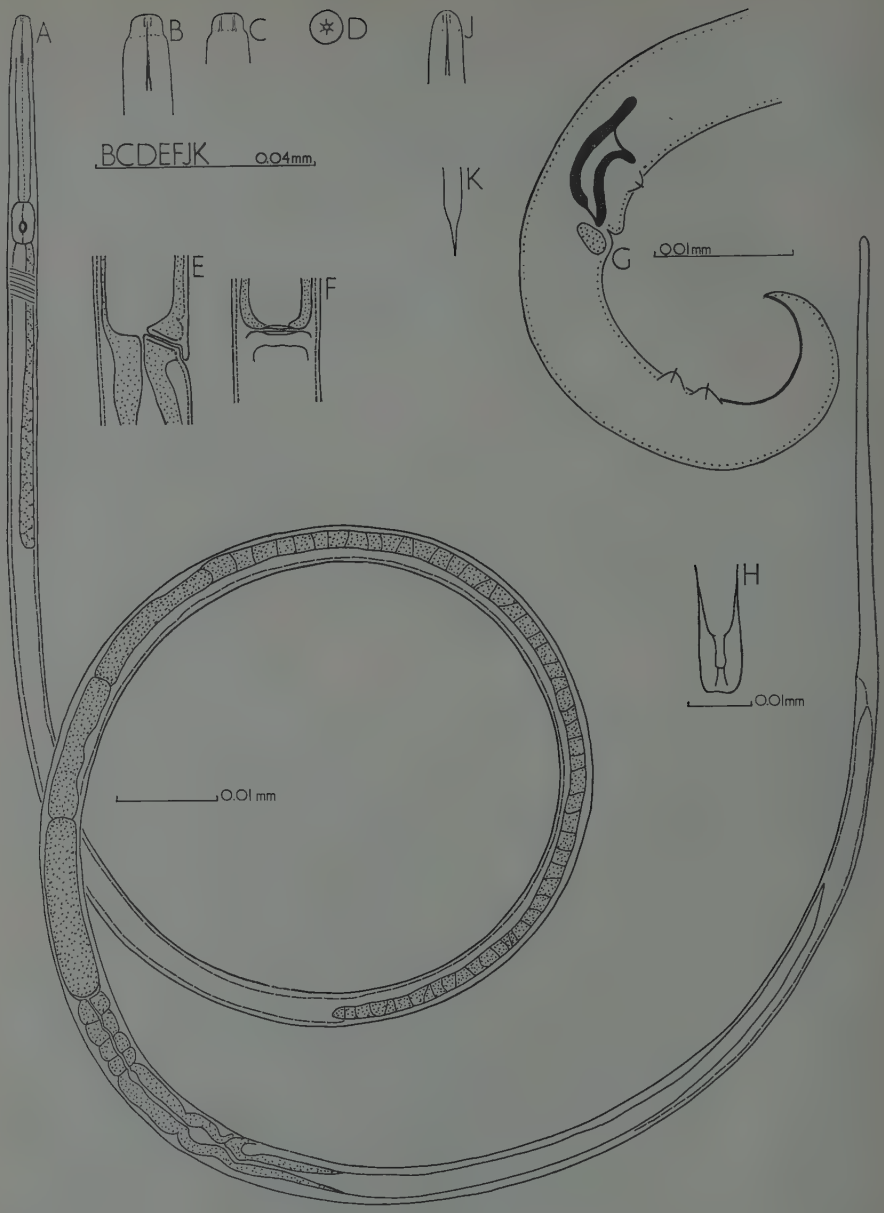

Fig. 1. *Rhadinaphelenchus cocophilus* (Cobb, 1919), n. comb.
A female; B sagittal section of head end; C surface view of head end; D end-on view of head; E sagittal section through vulva; F ventral view of vulva; G lateral view of male tail, greatly magnified; H ventral view of male tail; J head end of pre-adult larva; K tail end of pre-adult larva.

tion artifact in *Aphelenchoides winchesi*). Dorsal oesophageal gland opens midway between valve plates and anterior border, subventral glands just behind valve plates. Procorpus faintly, though easily, distinguishable in living specimens, is cylindrical with a slight constriction between its base and the median bulb. Oesophageal glands contained in a characteristic lobe overlapping intestine; in the specimens studied overlap appeared ventral or ventro-lateral. The lobe is slightly longer than procorpus; in living nematodes it is very difficult to discern, in fixed specimens it is impossible. Nerve ring visible, about a bulb-length behind median bulb and of about that width. Excretory pore about two bulb-lengths behind bulb.

Female: Vulva, at about two-thirds body-length, is covered by a flap of cuticle (Figs E & F) and leads into the vagina which slopes somewhat forward and almost halfway across the body. Anterior gonad functional, posterior a long post-vulval-sac reaching to about three-quarters of vulva-anus distance. Just beyond vulva, walls of post-vulval-sac are thick and probably represent part of the uterus, but remainder has very thin walls and the whole structure usually contains a few sperms. Anterior gonad consists of a short thick-walled uterus in two sections (Fig. A), leading to a portion made up of four rows of cells, probably equivalent to the quadricolumella of Wu (1958). Beyond this is the oviduct the thin walls of which are obscured when it contains developing eggs. The ovary itself consists of a single row of developing oocytes stretching well forward. Rectum about one and a half anal-body-widths long, flattened dorsoventrally, very difficult to see in lateral view. Anus a transverse slit about a quarter anal-body-width long (half according to Lordello & Zamith, 1954), reasonably visible, even in mounted specimens. Beyond the anus the body narrows slightly and then forms the long, gradually tapering, digitate tail, about fifteen anal-body-widths long. The end is rounded.

Male: Its most striking feature is the tail which, in dead specimens, curves ventrally to about four-fifths of a circle, bearing a terminal, bursal flap (Fig. G & H) which, in lateral view, shows as a line that remains sharp even when the focus of the microscope is varied. Tail bears two pairs of subventral, mucronate papillae just anterior to the origin of the bursal flap and there is another pair of similar papillae a little anterior to the cloacal opening. Spicules paired; dorsal limb (about 11 μ long) of each is prolonged forwards as a rounded apex; the anterior end of each ventral limb (about 7 μ long) curves ventrally as a short rostrum (Fig G) and is prolonged posteriorly more than the dorsal limb. Limbs are joined together posteriorly by a thin septum and anteriorly by a narrow transverse bar. Posterior to the spicules and beyond the cloaca is an apophysis, which seems to serve as a gubernaculum but is made of different material from the spicules. Testis single, is outstretched and reaches over halfway up the body.

Larva: Pre-adult larvae have conical, rounded heads, without annules and not set off from the body (Fig. J). The tail is slightly shorter than that of the female — about ten anal-body-widths long — and has a sharp end with a mucron (Fig. K).

Acknowledgement. I am very grateful to Dr. David Fenwick, Director, Red Ring Research Scheme, Trinidad for sending the live material on which the observations were made.

ZUSAMMENFASSUNG

Rhadinaphelenchus cocophilus *(Cobb, 1919) n. comb., der Nematode, verbunden mit der „Red-ring" Krankheit der Kokospalme*

Aphelenchoides cocophilus wird aus frischem Material neubeschrieben und in die neuaufgestellte Gattung *Rhadinaphelenchus* als *Rhadinaphelenchus cocophilus* (Cobb, 1919) n. comb. eingereiht. Der Nematode ist äusserst lang und schlank (a $\frac{\frown}{}$ 100) und durch eine läppchenartige terminale Bursa beim Männchen sowie durch ein Oberhautläppchen über der Vulva des Weibchens charakterisiert.

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A STUDY OF THE APPARENT DECAY OF EGGS WITHIN CYSTS OF *HETERODERA SCHACHTII* SCHMIDT AND *H. GÖTTINGIANA* LIEBSCHER, AND OF FREE LARVAE IN SOIL

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In hatching tests on cysts of *Heterodera* spp., the number of residual eggs, that is eggs from which the larvae have not emerged during the test, is sometimes estimated and added to the number of emerged larvae to calculate the initial egg content of the cysts. The initial egg content calculated in this manner often differs greatly between batches of cysts selected at random from a common source. In the course of routine hatching tests it was observed that the initial egg content

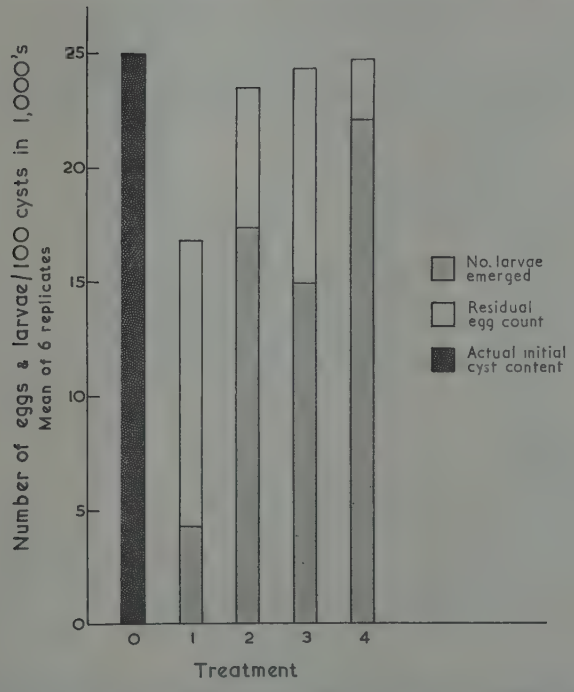

Fig. 1. Differences in the calculated initial egg content of cysts in a hatching test (duration 11 weeks) with *Heterodera schachtii*. Treatment 1, water hatch; treatments 2-4, hatch in beet diffusate

appeared to be directly correlated with the number of larvae that hatched over a long period. Fig. 1 shows the results of a hatching test with *H. schachtii* Schm. in water and in diffusate, with other superimposed treatments irrelevant in the present context. Treatment 1 is the emergence in water and treatments 2-4 that in beet diffusate. The test lasted 11 weeks. Far fewer larvae emerged in water than in diffusate and the total eggs and larvae that could be accounted for in this experiment with fully randomised batches of cysts were significantly lower in the cysts from which few larvae had emerged.

These results suggested that the decline in egg content of cysts in soil under fallow or non-host crops might be partly due to the decay of eggs within cysts. Field experiments that would answer this question are difficult to devise, and so the laboratory experiments which follow were used to show what might happen in the field.

When studying the emergence of larvae from cysts in soil, the free larvae are extracted and counted at regular intervals, and the fall in cyst contents over the same period estimated. The survival of larvae in the soil was also studied because an appreciable death rate would lower the numbers that are extracted and underestimate larval emergence.

EXPERIMENTS AND RESULTS

To test the effect of leaving cysts in water (part of the normal procedure in hatching tests), batches of 100 cysts of *H. schachtii* were placed in tap water in

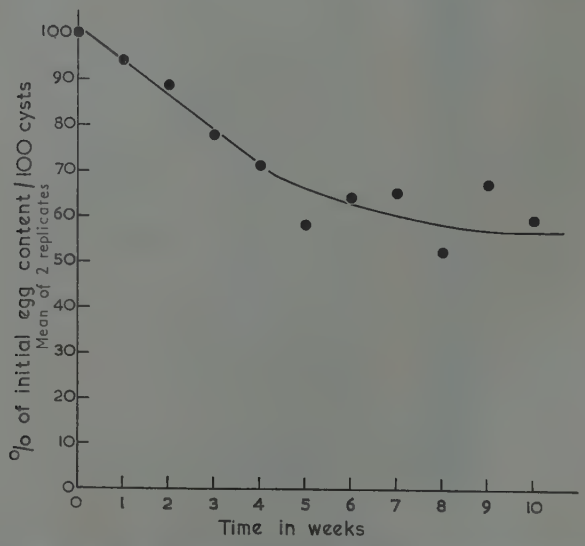

Fig. 2. The calculated initial egg content, that is the sum of residual egg content and larval emergence counts, for batches of 100 cysts of *Heterodera schachtii*, expressed as a percentage of the actual initial egg content, over a period of 10 weeks.

watch glasses for from 1-10 weeks at 25°C. At weekly intervals, two batches were removed and their egg contents estimated (Fig. 2). The larvae that emerged were counted and added to the residual egg count to estimate the total cyst contents. These appeared to decrease over the first 5 weeks, but then the counts became much more irregular, with greater variability between replicates. The experiment was therefore repeated in a modified form. Ten-fold replication was used and the contents estimated at 2-weekly intervals over 8 weeks (Fig. 3). Once again there

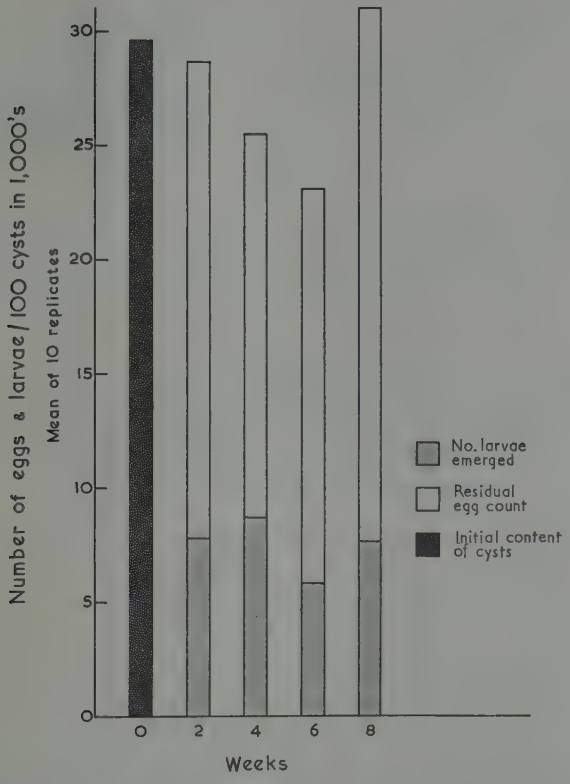

Fig. 3. Residual egg content and larval emergence counts, the sum of which is the calculated initial egg content, for batches of 100 cysts of *Heterodera schachtii* over a period of 8 weeks.

was an apparent fall in total contents over the first 6 weeks, but this was not statistically significant. The differences which appeared in the first experiment, although they were significant, may have been fortuitous or attributable to the few replicates used.

To find whether eggs also disappeared from cysts in soil, batches of 100 cysts of *H. schachtii* were mixed with a little sand and enclosed in nylon bags. These were placed in the centre of pieces of polythene tubing 0.8 cm bore and 4 cm long,

filled with unsterilised field soil sieved to a crumb size of 250-500 μ. The ends of the tubes were closed with rubber tubing (Fig. 4a). Three-fold replication was used and the cysts given three different moisture regimes, namely air dry, saturated and moderately wet (corresponding to field capacity). The last was achieved by equilibrating the soil to a suction of 20 cm of water, giving a moisture content of 15-20%, by Wallace's method (personal communication) as follows.

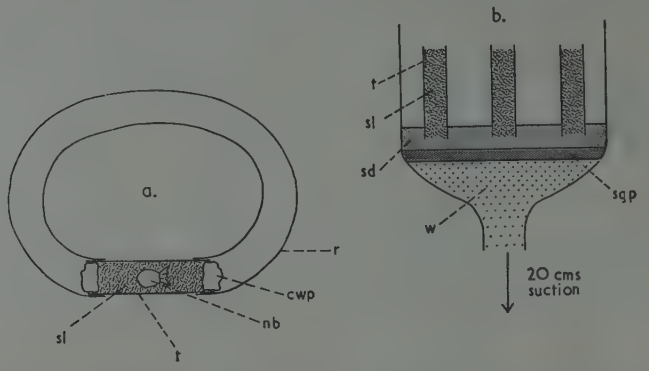

Fig. 4. a. Diagram of apparatus used to study the effects of various soil conditions on the egg content of cysts of *Heterodera schachtii* and *H. göttingiana*. cwp, cotton wool plug; nb, nylon bag containing cysts; r, rubber tubing; sl, soil; t, polythene tube.
b. Apparatus for producing a controlled moisture content in the soil in polythene tubes, using a sintered glass funnel. sd, sand (particle size 250-500 μ); sgp, sintered glass plate; sl, soil (particle size 250-500 μ); t, polythene tube; w, water.

The empty tubes, open at both ends, were pushed a short way into sand in a sintered glass funnel, filled with soil and then the required suction applied (Fig. 4b). The soil in the tubes held the corresponding moisture content when removed from the sand. The cysts were left up to 8 weeks, four batches from each treatment being examined at 2-weekly intervals (Fig. 5a). The number of larvae which hatched spontaneously was estimated simultaneously with the removal of the cysts. They were extracted from the soil by repeated decanting, followed by sieving the suspension through seven sieves of 55 μ aperture, a method which was satisfactory for the small quantities of soil used. The larval counts were then added to the residual egg counts, as before, to estimate total cyst contents. The contents of the cysts did not decrease significantly under any of the conditions, the low figure for the sixth week under 20 cm suction probably being fortuitous.

To avoid estimating larval emergence, the experiment was repeated using cysts of *H. göttingiana* Lieb., which have a negligible water hatch in the laboratory (Fig. 5b). Again the egg content of the cysts did not fall significantly over a period of 8 weeks in any of the treatments.

The polythene tube method was also used to test the rate of decay of free larvae in soil with a moisture content of 15-20 % (20 cm suction). Hatched larvae of *H. schachtii* were added in a little water to the soil in the tubes before the suction

was applied. There were four replicates and the larvae were extracted at weekly intervals over 8 weeks by the method described above. The first extraction was made on the same day that larvae were added and this count was regarded as the initial inoculum level (Fig. 6). The number of larvae recovered decreased rapidly

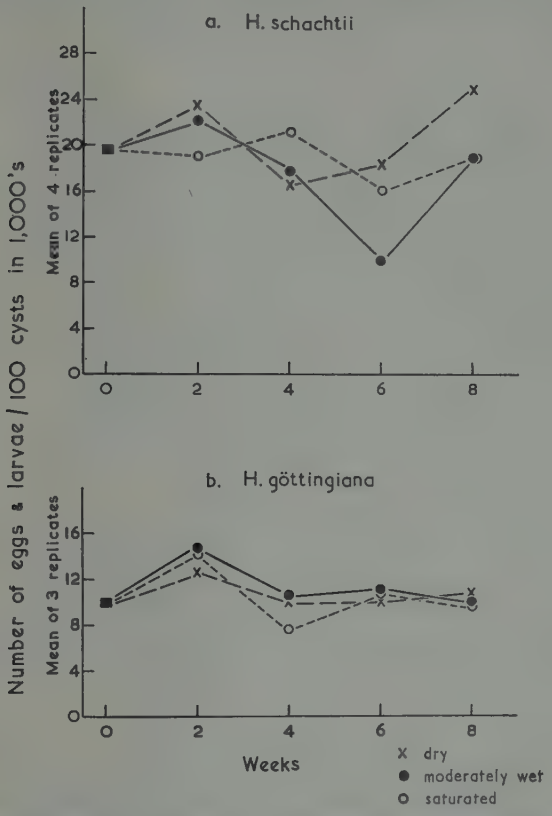

Fig. 5. Egg content of batches of 100 cysts of a. *Heterodera schachtii* and b. *H. göttingiana* kept in soil with different moisture regimes for up to 8 weeks. Moisture content of the moderately wet soil, 15-20%. The initial egg content is based on the mean of (a) 8 replicates and (b) 10 replicates.

during the first 3 weeks, after which the decline became much slower. After 8 weeks, however, 92.5% of the larvae had disappeared. A logarithmic transformation of the data shows that the rate of decay was steady at 34%/week. During the course of the experiment, a large proportion of the larvae became almost transparent, although many of these could still move. Many of the dead were filled with fungal spores.

When this experiment was repeated using sintering glass funnels (4 cm dia.) instead of polythene tubes, the decay rate (8%/week) was much slower (Fig. 6). The same soil was used, 1 cm deep in the funnels, with 20 cm suction as before.

There were three replicates and counts were made at 2-weekly intervals over 6 weeks. The volume of soil in the funnels was, however, four times that in the tubes, but the same number of larvae was used. Moreover, there was a much greater surface for air exchange in the funnels, and these factors may have contributed to the difference in decay rates.

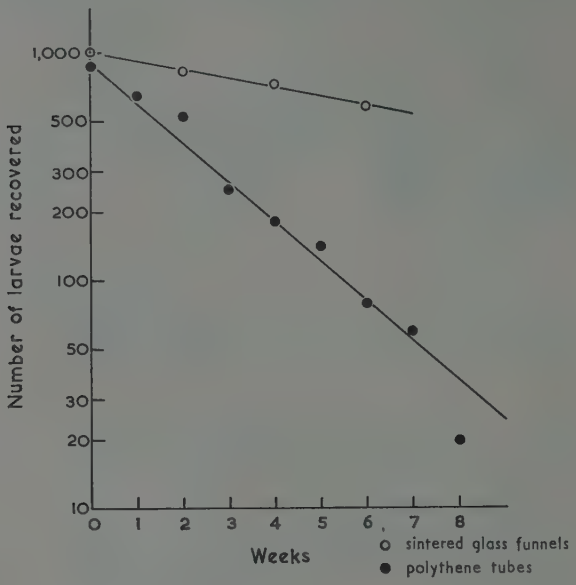

Fig. 6. Logarithmic relationship between numbers of larvae of *Heterodera schachtii* recovered from soil with a moisture content of 15-20% and time, under two sets of conditions.

DISCUSSIONS

Cooper (1953), Jones (1956), Hijner (1956) and Brown (1958) calculated the rate of decay of populations of *Heterodera* spp. in terms of eggs/g soil by recovering cysts from soil and estimating their egg content. Ellenby (1955), in pot experiments, placed batches of cysts in small wire baskets so that they could be easily recovered after given periods, and Wallace (1956) used this method to find the decrease in cyst contents. Wallace (1956) and Hesling (1958) used cyst contents to measure the rate at which larvae emerge from cysts under various conditions in the soil. The methods used by the above workers make it impossible to say how much of the fall is attributable to emergence and how much, if any, to decay of eggs within the cysts, or perhaps to predation by soil arthropods on the cysts. Doncaster (1957), however, showed in a pot experiment with *H. cruciferae* Franklin that about 7% of the cysts recovered were apparently damaged by predators.

Although the results of my experiments on the disappearance of eggs from

cysts are inconclusive, they provide little evidence that many eggs normally decay in cysts in soil. However, eggs do seem to be lost in some circumstances, perhaps because the microflora present occasionally causes epidemics. This might be especially liable to happen under the conditions of hatching tests, where cysts are in close contact with each other. But, in general, there seems little doubt that, with *H. schachtii* at least, hatching of larvae is responsible for the majority of any loss of cyst contents in soil.

Several attempts have been made previously to discover the length of time that free larvae of *Heterodera* can survive in the soil in the absence of a host plant. Berliner & Busch (1914) found larvae still mobile after 14 days in water and concluded that the stored food reserves in the intestinal cells would probably be sufficient for a much longer period. Baunacke (1922) observed that after keeping larvae of *H. schachtii* in water in the laboratory for 5-6 weeks, some were still able to move. The first systematic attempt to determine the length of survival was made by Nebel (1926), who kept larvae of *H. schachtii* in pots of sterilised soil in an unheated room for 7-379 days. He estimated the number still living by growing rape seedlings in the soil after various periods and counting the number of larvae that invaded the roots. He never recovered more than about 10% of the larvae he added to the soil, even after only 7 days, but at the end of a year he still recovered 1-3% of his initial inoculum. Franklin (1937) inoculated soil with larvae of *H. rostochiensis* Woll. at the rates of 1.5 and 7 larvae/ml soil and kept them either outside or indoors for 3-16 months. Potatoes were grown in samples taken at 2-monthly intervals. The most cysts found on the roots of any plant was seven. No cysts were found from soil kept outside after 9 months, and none from that indoors after 11 months, although a few second stage larvae were recovered from the roots of plants grown in soil after 16 months indoors. Bergeson (1959) studied the influence of temperature on the survival of larvae of *Meloidogyne* spp. in soil, using the galling of host roots as an estimate of survival. He found that, at 60-70°F, only 10-20% of the larvae still survived after 2 months.

All these workers based their estimates of the survival of larvae in soil on the ability of the larvae to invade and develop in a host plant after a period of starvation. This is, of course, the ultimate criterion, but it involves additional sources of error and variability, so that it cannot be used as an absolute measure of the rate at which larvae die in the soil. The extraction of larvae from the soil shows that populations steadily decline in the absence of a host plant, but that a small proportion may survive for a considerable time, as has been demonstrated by earlier workers on this subject. The larvae are clearly very sensitive to soil conditions, which largely determine the rate at which they succumb.

ZUSAMMENFASSUNG

Über ein augenscheinliches Absterben von Eiern innerhalb der Zysten von Heterodera schachtii *Schmidt und* H. göttingiana *Liebscher und freier Larven im Boden*

In Versuchsreihen mit zufällig ausgewählten Zysten zeigten Schätzungen des Gesamtzysteninhalts, bestehend aus der Zahl der schlüpfenden Larven und der zurückbleibenden Eier, zuweilen grosse Unterschiede. Diese sind am grössten, wenn auch das Schlüpfmass stark schwankt. Sie deuten an, dass Eier innerhalb der Zysten zugrundegehen können, wenn die Larven im Versuch nicht rechtzeitig schlüpfen. Aus Versuchen mit Zysten von *Heterodera schachtii,* die in Wasser zwei Monate gehalten wurden und aus Zysten von *H. schachtii* und *H. göttingiana,* die sich in Boden von verschiedenem Feuchtigkeitsgehalt befanden, konnte keine Gewissheit darüber erlangt werden. Der grösste Verlust des Zysteninhalts im Boden wird dem Larvenschlupf zuzuschreiben sein. Die anwesende Mikroflora kann gelegentlich, besonders unter den Bedingungen des Schlüpfversuchs, Epidemien hervorrufen. Larven von *H. schachtii* nehmen auch im Boden, der einen der Feldkapazität entsprechenden Feuchtigkeitsgehalt hat, beim Fehlen einer Wirtspflanze zahlenmässig schnell ab. Sie sind gegenüber Bodenbedingungen sehr empfindlich, die das Mass des Absterbens bestimmen. In den beschriebenen Versuchen wurden die freien Larven aus einer kleinen Bodenmenge durch Dekantieren und Sieben der Suspension extrahiert.

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THE CLASSIFICATION OF THE APHELENCHOIDEA FUCHS, 1937

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Since the publication of *Soil and freshwater nematodes* (T. GOODEY, 1951) papers concerned with the classification of the Aphelenchoidea have been published by ALLEN (1952), PARAMONOV (1953), FRANKLIN (1955), WACHEK (1955), RÜHM (1956), CHITWOOD (1958) and HOPPER & CAIRNS (1959), which is based on CHITWOOD (1958). In addition the paper by SKARBILOVICH (1947), although published before *Soil and freshwater nematodes* has to be considered in any discussion of the subject.

Some of the systems advocated are incomplete as they deal with part only of the superfamily, albeit at generic or specific level. CHITWOOD (1958) deals with the whole superfamily but only down to subfamily level.

From the published work and with *Aphelenchus* and *Aphelenchoides* (sensu T. GOODEY, 1951) from specimens as well, the following classification, which includes brief definitions of the various groups and comments, has been prepared.

Order: TYLENCHIDA (Marcinowski, 1909) Thorne, 1949

Superfamily: Aphelenchoidea (Fuchs, 1937) Thorne, 1949

[Aphelenchoidea Fuchs, 1937 was a subdivision of subfamily Tylenchinae]

Definition: Dorsal oesophageal gland emptying into lumen of oesophagus within the median muscular bulb anterior to the crescentic valve plates, or in that position when no median bulb is present.

Family: APHELENCHIDAE (Fuchs, 1937) Steiner, 1949.

Definition: Oesophageal glands contained in a lobe lying separate in the body and joining the alimentary canal behind the bulb. Male with bursa supported by 4 pairs of caudal rays. Spicules long and narrow; gubernaculum present.

Subfamily: Aphelenchinae (Fuchs, 1937) Schuurmans Stekhoven & Teunissen, 1938 redundant.

Genus: *Aphelenchus* Bastian, 1865
syn. *Isonchus* Cobb, 1913
Type species: *Aphelenchus avenae* Bastian, 1865

syn. *A. agricola* de Man, 1881
A. agricola Maupas, 1900
A. *(Paraphelenchus) maupasi* Micol., 1922
P. maupasi (Micol., 1922) Micol., 1925

A. maupasi Micol., 1922 (Steiner, 1942)
A. cylindricaudatus (Cobb *in* Steiner, 1926) Steiner, 1931
Tylenchus cylindricaudatus Cobb *in* Steiner, 1926
Aphelenchus macrobolbus Steiner, 1942
Aphelenchoides solani Steiner, 1935
Isonchus radicicola Cobb, 1913

Description: see above.
The synonomising of all the above with *A. avenae* is done deliberately to call attention to the lack of detail adequate, by present day standards, to distinguish the various species that have been made.

Other species:

A. maximus Das, 1960
A. mirzai Das, 1960

These just-described species may be within the range of variation shown by *A. avenae*. The details given for them are meagre.

Family: APHELENCHOIDIDAE (Skarbilovich, 1947) Paramonov, 1953
Definition: Oesophageal glands forming a lobe lying separately, and apparently dorsally, in the body and joining the alimentary canal behind the bulb. Male tail usually without, sometimes with, slight bursal folds or terminal bursal flap. One, two or three pairs of subventral (very rarely subdorsal) caudal papillae, and sometimes pairs of glandular subventral papillae, present. Spicules shaped more or less like a rosethorn or derived from that shape. No gubernaculum.

Subfamilies: Aphelenchoidinae Skarbilovich, 1947 and Parasitaphelenchinae Rühm, 1956 sink to redundancy level.

One of the criteria used in the separation of the genera of this family is the form of the spicules. There is a ventrally curved shaft formed of a dorsal and ventral limb, which appear to be separate but are actually joined terminally. The junction is not easy to see and many figures of aphelenchoid spicules do not show it. The two limbs are joined anteriorly by a transverse bar which may be prolonged ventrally into a more or less prominent rostrum, and the other end may also be prolonged together with the dorsal limb into a more or less prominent apex.

Genus: *Aphelenchoides* Fischer, 1894

syn. *Schistonchus* Cobb, 1927 (subg.)

Definition: Six fused, non-annulated, similar lips, slightly offset from body. Male tail without bursa, with one pair of approximately adanal and two pairs of postanal, ventro-submedian, caudal papillae. Spicules paired and shaped like rose thorns. Tails of both sexes never elongate filiform but short, more or less tapering, conical and frequently ending in one or more mucrones.

Type species: *Aphelenchoides kühnii* Fischer, 1894

The original description is very poor but is sufficient to characterise the form of the spicules and male tail.

Other species:

A. abyssinicus (Filipjev, 1931) Fil. & Sch. Stek., 1941
syn. *Aphelenchus abyssinicus* Filipjev, 1931
A. asterocaudatus Das, 1960
A. besseyi Christie, 1942
syn. *A. oryzae* Yokoo, 1948
A. bicaudatus (Imamura, 1931) Fil. & Sch. Stek., 1941
syn. *Aphelenchus bicaudatus* Imamura, 1931
A. blastophthorus Franklin, 1952
A. brevicaudatus Das, 1960
A. brevionchus Das, 1960
A. chamelocephalus (Steiner, 1926) Fil. & Sch. Stek., 1941
syn. *Aphelenchus chamelocephalus* Steiner, 1926
A. coffeae (Zimmerman, 1896) Steiner, 1937
syn. *Aphelenchus coffeae* Zimmerman, 1896
A. composticola Franklin, 1957
A. cyrtus Paesler, 1957
A. dactylocercus Hooper, 1958
A. fragariae (Ritzema Bos, 1891) Christie, 1932
syn. *Aphelenchus fragariae* Ritzema Bos, 1891
A. olesistus Ritzema Bos, 1893
Aphelenchoides olesistus (Ritzema Bos, 1893) Steiner, 1932
A. longicollis (Schwartz, 1911) Fil. & Sch. Stek., 1941
A. olesistus v. *longicollis* (Schwartz, 1911) T. Goodey, 1933
Aphelenchus olesistus v. *longicollis* Schwartz, 1911
A. pseudolesistus T. Goodey, 1928
Aphelenchoides pseudolesistus (T. Goodey, 1928) T. Goodey, 1933
A. goeldi (Steiner, 1914) Fil. & Sch. Stek., 1941
syn. *Aphelenchus goeldi* Steiner, 1914
A. hainanensis (Rahm, 1938) T. Goodey, 1951
syn. *Aphelenchus hainanensis* Rahm, 1938
A. helophilus (de Man, 1880) T. Goodey, 1933
syn. *Aphelenchus helophilus* de Man, 1880
A. elegans Micoletzky, 1914
A. kungradensis Karimova, 1957
A. limberi Steiner, 1936
A. longiurus Das, 1960
A. nonveilleri Andrassy, 1959
A. parietinus (Bastian, 1865) Steiner, 1932
syn. *Aphelenchus parietinus* Bastian, 1865
A. pusillus (Thorne, 1929) Fil. & Sch. Stek., 1941
syn. *Aphelenchus pusillus* Thorne, 1929
A. ritzemabosi (Schwartz, 1912) Steiner, 1932
syn. *Aphelenchus ritzemabosi* Schwartz, 1912
A. phyllophagus Stewart, 1921
A. ribes (Taylor, 1917) T. Goodey, 1923
Tylenchus ribes Taylor, 1917
Aphelenchoides ribes (Taylor, 1917) T. Goodey, 1933
A. sacchari Hooper, 1958
A. saprophilus Franklin, 1957
A. scalacaudatus Sudakova, 1958
A. singhi Das, 1960
A. sinodendroni Rühm, 1957
A. sphaerocephalus T. Goodey, 1953
A. spinosus Paesler, 1957

A. subtenuis (Cobb, 1926) Steiner & Buhrer, 1932
syn. *Aphelenchus subtenuis* Cobb, 1926
Aphelenchoides hodsoni T. Goodey, 1935
A. tagetae Steiner, 1941

The following is possibly a member of this genus but it is not clear whether Cobb had the same species as that inadequately described by Gasparrini. From a study of photographs of Cobb's original drawings it is clear that it is only the fore-part of the spear that is forked and on this phenomenon the subgenus *Schistonchus* is based.

A. caprifici (Gasparrini, 1871) Fil. & Sch. Stek., 1941

syn. *Aphelenchus (Schistonchus) caprifici* (Gasparrini, 1871) Cobb, 1927
Anguillola caprifici Gasparrini, 1871

Genus: *Seinura* Fuchs, 1931

Definition: Similar to *Aphelenchoides* in many respects but tails of both sexes are elongate, filiform and not short conical. Spicules have prominent rostrum and apex. The pattern of papillae on the male tail appears to be a preanal pair (or a single papilla) at the level of proximal ends of spicules, an adanal pair and one or two postanal pairs on the conical part of the tail. Many and possibly all of these species are predatory in habit.

Type species: *Seinura mali* Fuchs, 1931

syn. *Aphelenchoides mali* (Fuchs, 1931) T. Goodey,

Other species:

S. citri (Andrassy, 1957) n. comb.
syn. *Aphelenchoides citri* Andrassy, 1957
S. christiei nom. nov.
syn. *Aphelenchoides tenuicaudatus* of Christie, 1939
S. demani (T. Goodey, 1928) n. comb.
syn. *Aphelenchus demani* T. Goodey, 1928
Aphelenchoides demani (T. Goodey, 1928) T. Goodey, 1933
S. diversa (Paesler, 1957) n. comb.
syn. *Aphelenchoides winchesi* v. *diversus* Paesler, 1957
S. elmiraensis (v. d. Linde, 1938) n. comb.
syn. *Aphelenchoides elmiraensis* v. d. Linde, 1938
S. filicaudata (Christie, 1939) n. comb.
syn. *Aphelenchoides winchesi* v. *filicaudatus* Christie, 1939
S. linfordi (Christie, 1939) n. comb.
syn. *Aphelenchoides linfordi* Christie, 1939
S. longicaudata (Cobb, 1893) n. comb.
syn. *Aphelenchus longicaudatus* Cobb, 1893
Aphelenchoides longicaudatus (Cobb, 1893) T. Goodey, 1933
S. oahuënsis (Christie, 1939) n. comb.
syn. *Aphelenchoides oahuënsis* Christie, 1939
S. oliveirae (Christie, 1939) n. comb.
syn. *Aphelenchoides oliveirae* Christie, 1939
S. oswegoensis (v. d. Linde, 1938) n. comb.
syn. *Aphelenchoides oswegoensis* v. d. Linde, 1938
S. oxura (Paesler, 1957) n. comb.
syn. *Aphelenchoides oxurus* Paesler, 1957
S. tenuicaudata (de Man, 1895) n. comb.

syn. *Aphelenchus tenuicaudatus* de Man, 1895
Aphelenchoides tenuicaudatus (de Man, 1895) T. Goodey, 1933
S. winchesi (T. Goodey, 1927) n. comb.
syn. *Aphelenchus winchesi* T. Goodey, 1927
Aphelenchoides winchesi (T. Goodey, 1927) T. Goodey, 1933

Probably belonging to this genus:

S. hyderabadensis (Das, 1960) n. comb.
syn. *Aphelenchoides hyderabadensis* Das, 1960
S. mucronatus (Paesler, 1946) n. comb.
syn. *Aphelenchoides mucronatus* Paesler, 1946
S. speciosus (Andrassy, 1958) n. comb.
syn. *Aphelenchoides speciosus* Andrassy, 1958

Genus: *Megadorus* n.g.

Definition: The chief features are the massive spear with large basal knobs, the anterior part of the oesophagus is wide and tapers rapidly to a thin cylinder before joining the large, prominent, oval-rounded, median bulb. The tail is short conical, without mucro. Only the female is known.

Type species: *Megadorus megadorus* (Allen, 1941) n. comb.
syn. *Aphelenchoides megadorus* Allen, 1941

No other species.

Genus: *Tylaphelenchus* Rühm, 1956

Definition: Offset head has six, equal lips each higher than wide. Spear 10-11 μ long, anterior pointed part only half the length of shaft, spear lumen narrow, basal knobs well-formed, offset. Tails of both sexes conical. Male tail with one preanal and one postanal pair of papillae and two tiny pairs of subterminal, subventral glandular papillae. Spicules with ventral flange and small rostrum, apex variable. Insect associates.

Type species: *Tylaphelenchus leichenicola* Rühm, 1956

Other species: *T. ipidicola* (Rühm, 1956) n. comb.
syn. *Aphelenchoides (Aph.) ipidicola* Rühm, 1956

Genus: *Laimaphelenchus* Fuchs, 1937

Definition: Characterised by the possession of four (? sometimes three) stalked and fringed tubercles terminating the female tail. Male tail similar or conoid with or without mucrones, a slight bursa may also be present. Inhabits mosses and lichens or associates of insects.

Type species: *Laimaphelenchus penardi* (Steiner, 1914) Fil. & Sch. Stek. 1941
syn. *Aphelenchus penardi* Steiner, 1914
Aphelenchoides penardi (Steiner, 1914) McBeth, 1937

Other species:

L. lignophilus (Körner, 1954)
syn. *Aphelenchoides lignophilus* Körner, 1954
L. moro Fuchs, 1937
L ulmi Khan, 1960

Genus: *Bursaphelenchus* Fuchs, 1937

syn. *Aphelenchoides (Bursaphelenchus)* (Fuchs, 1937) Rühm, 1956

Definition: Female vulva not covered by cuticular flap. Male with terminal bursal flap originating well back on tail where it begins to narrow to its tip. Spicules not fused. No apophysis. The species are insect associates.

Type species: *Bursaphelenchus piniperdae* Fuchs, 1937

Other species:

B. conjunctus (Rühm, 1956) n. comb.
syn. *Aphelenchoides (B.) conjunctus* of Rühm, 1956
nec *Parasitaphelenchus conjunctus* Fuchs, 1930
B. chitwoodi (Rühm, 1956) n .comb.
syn. *Aph. (B.) chitwoodi* Rühm, 1956
B. crenati (Rühm, 1956) n. comb.
syn. *Aph. (B.) crenati* Rühm, 1956
B. cryphali (Fuchs, 1930) n. comb.
syn. *Parasitaphelenchus cryphali* Fuchs, 1930
Aph. (B.) cryphali (Fuchs, 1930) Rühm, 1956
B. eggersi (Rühm, 1956) n. comb.
syn. *Aph. (B.) eggersi* Rühm, 1956
*B. eidmanni (*Rühm, 1956) n. comb.
syn. *Aph. (B.) eidmanni* Rühm, 1956
B. eremus (Rühm, 1956) n. comb.
syn. *Aph. (B.) eremus* Rühm, 1956
B. eucarpus (Rühm, 1956) n. comb.
syn. *Aph. (B.) eucarpus* Rühm, 1956
B. fraudulentus (Rühm, 1956) n. comb.
syn. *Aph. (B.) fraudulentus* Rühm, 1956
B. idius (Rühm, 1956) n. comb.
syn. *Aph. (B.) idius* Rühm, 1956
B. incurvus (Rühm, 1956) n. comb.
syn. *Aph. (B.) incurvus* Rühm, 1956
B. nuesslini (Rühm, 1956) n. comb.
syn. *Aph. (B.) nuesslini,* Rühm, 1956
B. poligraphi Fuchs, 1937
syn. *Aph. poligraphi* (Fuchs, 1937) T. Goodey, 1951
B. ratzburgii (Rühm, 1956) n. comb.
syn. *Aph. (B.) ratzburgii* Rühm, 1956
B. sachsi (Rühm, 1956) n. comb.
syn. *Aph. (B.) sachsi* Rühm, 1956
B. steineri (Rühm, 1956) n. comb.
syn. *Aph. (Aph.) steineri* Rühm, 1956
B. sychnus (Rühm, 1956) n. comb.
syn. *Aph. (B.) sychnus* Rühm, 1956
B. xerokartus (Rühm, 1956) n. comb.
syn. *Aph. (B.) xerokartus* Rühm, 1956

The following two species probably belong to this genus and Rühm (1956) suggests they may be synonymous.

B. conurus (Steiner, 1932) n. comb.
syn. *Aph. conurus* Steiner, 1932
B. talonus (Thorne, 1935) n. comb.
syn. *Aph. talonus* Thorne, 1935
Aph. (B.) talonus Thorne, 1935 (Rühm, 1956)

Genus: *Rhadinaphelenchus* J. B. Goodey, 1960

Definition: Very slender nematodes (a $\triangleq$ 100); female with vulval flap, long post-vulval uterine sac, long digitate tail; male with terminal bursal flap; apophysis present beyond spicules. Associated with Red-ring disease of coconut.

Type species: *Rhadinaphelenchus cocophilus* (Cobb, 1919) J. B. Goodey, 1960
syn. *Aphelenchus cocophilus* Cobb, 1919
A. (Chitinoaphelenchus) cocophilus Cobb, 1919 (Micol., 1922)
Aphelenchoides cocophilus (Cobb, 1919) T. Goodey, 1933
Chitinoaphelenchus cocophilus (Cobb, 1919) Chitwood in Corbett, 1959

No other species.

Genus: *Cryptaphelenchus* (Fuchs, 1937) Rühm, 1956
syn. *Parasitaphelenchus (Cryptaphelenchus)* Fuchs, 1937
P. (Steineria) Fuchs, 1937

Definition: Adults about 0.5 mm. long and not slender (a = about 20). Lips rounded and forming a flat cap, not offset. Spear usually less than 10 μ; its lumen not visible. Female rectum and anus usually lacking; posterior end of gut forming a rounded, blind sac. No post-vulval sac. Male tail with one pair just preanal and one pair well postanal of caudal papillae. Spicule shafts well curved with a prominent, narrow rostrum and rounded apex which continues the axis of the curving transverse bar. Posterior to the spicule tips is a structure which, according to Rühm (in litt.), is not a gubernaculum. Insect associates.

Type species: *Cryptaphelenchus macrogaster macrogaster* (Fuchs, 1915), Rühm, 1956
syn. *Tylenchus macrogaster* Fuchs, 1915
Parasitylenchus macrogaster (Fuchs, 1915) Fuchs, 1930
P. (Cryptaphelenchus) macrogaster (Fuchs, 1915) Fuchs, 1937
Aphelenchoides macrogaster (Fuchs, 1915) T. Goodey, 1951

Other species:

C. cryptus Rühm
C. hectographi Rühm *in* Rühm & Chararas, 1957
C. koerneri Rühm, 1956
C. latus (Thorne, 1935) Rühm, 1956
syn. *Aphelenchoides latus* Thorne, 1935
C. leptocaudus Rühm, 1956
C. macrogaster acuminati Rühm, 1956
C. macrogasteroides macrogasteroides Rühm, 1956

C. macrogasteroides villosi Rühm, 1956
C. malpighius bidentati Rühm, 1956
C. malpighius malpighius (Fuchs, 1937) Rühm, 1956
syn. *Parasitaphelenchus (C.) malpighius* Fuchs, 1937
Aphelenchoides malpighius (Fuchs, 1937) T. Goodey, 1951
C. minutus (Fuchs, 1930) Rühm, 1956
syn. *Parasitaphelenchus minutus* Fuchs, 1930
P. (C.) minutus Fuchs, 1930 (Fuchs, 1937)
Aph. minutus (Fuchs, 1930) T. Goodey, 1951
C. piceae Rühm, 1956
C. piceoides Rühm, 1956
C. poligraphi Rühm, 1956
C. viktoris (Fuchs, 1937) Rühm, 1956
syn. *Parasitaphelenchus (Steineria) viktoris* Fuchs, 1937
Aph. viktoris (Fuchs, 1937) T. Goodey, 1951

The following species probably belongs here:

C. pygmaeus (Fuchs, 1930) Rühm, 1956
syn. *Parasitaphelenchus pygmaeus* Fuchs, 1930
P. (C.) pygmaeus Fuchs, 1930 (Fuchs, 1937)
Aph. pygmaeus (Fuchs, 1930) T. Goodey, 1951.

Genus: *Cryptaphelenchoides* n.g.

Definition: Head offset; lips equal, rounded, slightly higher than wide. Spear 16 μ, with wide lumen and insignificant basal knobs. Procorpus relatively short, median bulb large, elongate-round, longer than body-width at that level. Large valves in second half of the bulb. Anus and rectum visible but gut ends in a blind sac that projects well into the female tail. Female tail conical to a rounded tip. Vulva posterior; vagina at right angles to the body; post-vulval sac short. Male tail bluntly rounded with one preanal (head of spicule level), one adanal (post-anal) and one postanal (mid caudal) pair of subventral papillae. Spicules much curved ventrally with prominent rostrum and large apex which continues the line of the shafts. An apophysis is connected with the distal ends of the spicules. Insect associate.

Type species: *Cryptaphelenchoides macrobulbosus* (Rühm, 1956) n. comb.
syn. *Aphelenchoides (Aph.) macrobulbosus* Rühm, 1956

See Rühm, (1956) p. 215 for figures.
No other species.

Genus: *Ektaphelenchus* (Fuchs, 1937) Rühm, 1956
syn. *Parasitaphelenchus (Ektaphelenchus)* Fuchs, 1937

Definition: Long slender nematodes. Head offset, cap-like, the six individual lips very distinctly separate from one another, the laterals narrower and projecting

slightly in front of the others. Spear not less than 15 μ long with wide lumen. Median bulb rectangular. Rectum and anus probably non-functional, so that the hind end of the gut is a blind diverticulum. Male tail with one preanal and one or two postanal pairs of subventral papillae and occasionally a median, ventral, preanal papilla. Spicules ventrally curved with prominent rostrum and large prominent apex continuing the line of the shafts. Insect associates.

Type species: *Ektaphelenchus hylastophilus* (Fuchs, 1930) n. comb.
syn. *Parasitaphelenchus hylastophilus* f. *ateri* Fuchs, 1930
P. (E.) hylastophilus f. *ateri* Fuchs, 1930 (Fuchs, 1937)
P. hylastophilus f. *cunicularii* Fuchs, 1930
P. (E.) hylastophilus f. *cunicularii* Fuchs, 1930 (Fuchs, 1937)
E. cunicularii (Fuchs, 1930) Rühm, 1956

Rühm (1956) names *E. tuerkorum* as a type species but is wrong for the subgenus already had a type which cannot change.

Other species:

E. alni (Steiner, 1942) Rühm, 1956
syn. *Aphelenchoides alni* Steiner, 1942
E. amitini (Fuchs, 1937) Rühm, 1956
syn. *Parasitaphelenchus (Cryptaphelenchus) amitini* Fuchs, 1937
Aph. amitini (Fuchs, 1937) T. Goodey, 1951
E. betulae Rühm, 1956
E. dendroctoni Rühm, 1956
E. goffarti Rühm, 1956
E. macrostylus Khan, 1960
E. obtusus Massey, 1956
E. scolyti Rühm, 1956
E. tenuidens (Thorne, 1935) Thorne *in* Rühm, 1956
syn. *Aph. tenuidens* Thorne, 1935
E. tuerkorum Rühm, 1956
E. typographi (Fuchs, 1930) Rühm, 1956
syn. *Parasitaphelenchus typographi* Fuchs, 1930
P. (E.) typographi Fuchs, 1930 (Fuchs, 1937)
E. zwölferi Rühm, 1957

Possibly also belonging here are:

E. brachycephalus (Thorne, 1935) n. comb.
syn. *Aph. brachycephalus* Thorne, 1935
E. stammeri (Körner, 1954) n.c.
syn. *Aph. stammeri* Körner, 1954

Genus: *Parasitaphelenchus* Fuchs, 1930

Definition: Body very long and slender. Six similar, rounded lips, clearly offset from the body and one another, each as high as wide. Vulva well posterior (85%) with protuberant lips. Post-vulval sac usually long. Spicules fused along their shafts forming a shovel-like structure; rostrum prominent and apex well-developed as a continuation of the line of the shafts. Male tail short, conical, sometimes with offset tip and with small, fold-like or terminal, flap-like bursa and three pairs

of conspicuously large, variably placed, caudal papillae. Larvae are parasites of bark beetle, adults free-living.

Type species: *Parasitaphelenchus uncinatus* (Fuchs, 1929) Fuchs, 1930

syn. *Tylenchus uncinatus* Fuchs, 1929
P. uncinatus ateri (Fuchs, 1929) Fuchs, 1937
P. ateri (Fuchs, 1929, 1937) Rühm, 1956
Aphelenchoides uncinatus ateri (Fuchs, 1929, 1937) T. Goodey, 1951

Other species:

P. acroposthion (Steiner, 1932) Rühm, 1956
syn. *Aphelenchoides acroposthion* Steiner, 1932
P. acuminati Rühm, 1956
P. heterophallus (Steiner, 1934) Rühm, 1956
syn. *Aph. heterophallus* Steiner, 1934
P. oldhami Rühm, 1956
P. oregonensis (Steiner, 1942) Rühm, 1956
syn. *Aph. oregonensis* Steiner, 1942
P. papillatus Fuchs, 1937
syn. *Aph. papillatus* (Fuchs, 1937) T. Goodey, 1951
P. poligraphi (Fuchs, 1937) Rühm, 1956
syn. *P. uncinatus poligraphi* Fuchs, 1937
Aph. uncinatus poligraphi (Fuchs, 1937) T. Goodey, 1951
P. sexdentati (Fuchs, 1937) Rühm, 1956
syn. *P. uncinatus sexdentati* Fuchs, 1937
Aph. uncinatus sexdentati (Fuchs, 1937) T. Goodey, 1951
P. strongylocephalus Rühm, 1956

Possibly also belonging here:

P. gallagheri (Massey, 1960) n. comb.
syn. *Aphelenchoides gallagheri* Massey, 1960

Genus: *Peraphelenchus* Wachek, 1955

Definition: Spear 17-18 μ long, pointed anterior end forming a quarter only of length. Median bulb trapezoid. Female ventrally curved; vulva at 79%; ovary few-celled; post-vulval sac about a body-width long and serving as a spermatheca. Female tail shortly conical. Male corkscrew-shaped with a single spicule (perhaps a completely fused pair); without rostrum but with prominent, pointed apex. Male tail has two pairs of postanal, subventral, caudal papillae. All stages parasitic in species of burying beetles, *Necrophorus* (Silphidae). Fertilized female migrate via the host rectum and enter new host larvae.

Type species: *Peraphelenchus necrophori* Wachek, 1955

Genus: *Entaphelenchus* Wachek, 1955

Definition: Lips rounded, flattened and slightly offset. Vulva posterior (85% or more); ovary of a few cells only in free-living female; postvulval sac less than a body-width long. Female tail conical to a rounded tip. Male tail with one pair of preanal, two pairs of post-anal (mid tail and subterminal) subventral papillae and sometimes one pair of postanal, subdorsal, subterminal papillae. Spicules short and broad with prominent rostrum and large apex continuing the line of the shafts.

The fertilized female and larvae are parasites in the haemocoele of various Staphylinid beetles. The parasitic female doubles in size, the ovary develops considerably and extensive perivaginal cells fill the latter part of the body. Insect associates.

Type species: *Entaphelenchus oxyteli* Wachek, 1955

Other species:

E. aliantae Wachek, 1955
E. bledii Wachek, 1955
E. philonthi Wachek, 1955
E. platystethi Wachek, 1955
E. xantholini Wachek, 1955

Family: PARAPHELENCHIDAE (T. Goodey, 1951) n. grad.

Definition: Oesophageal glands contained within the confines of the post-bulbar region of the oesophagus. Male tail without bursa; with four or five pairs of caudal papillae; spicules long and narrow; gubernaculum present.
Subfamily: Paraphelenchinae T. Goodey, 1951 becomes redundant.

Genus: *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925
syn. *Aphelenchus (Paraphelenchus)* Micoletzky, 1922

Definition: Second oesophageal bulb spathulate. Female tail short, conical, sometimes with terminal mucron. Male tail with characteristic pattern of caudal papillae — Pair 1 (may be absent or a single median papilla) preanal, pairs 2, 3 and 4 subventral, 2 adanal, 3 mid-tail, 4 subterminal, pair 5 subterminal and subdorsal.

Type species: *Paraphelenchus pseudoparietinus* (Micol., 1922) Micol., 1925
syn. *Aphelenchus (P.) pseudoparietinus* Micol., 1922

Other species:

P. amblyurus Steiner, 1934
P. arenaceus T. Goodey, 1944
syn. *Aphelenchus agricola* of de Man, 1921
P. basili Das, 1960
P. myceliophthorus J. B. Goodey, 1958
P. tritici Baranovskaya, 1958

Genus: *Metaphelenchus* Steiner, 1943

Definition: Remarkably similar in all characters to *Aphelenchus avenae* but appearing to have a terminal oesophageal bulb. This genus needs to be found again and studied in detail to determine its validity.

Type species: *Metaphelenchus rhopalocercus* Steiner, 1943

Other species:

M. micoletzkyi (Steiner, 1941) Steiner, 1943
syn. *Paraphelenchus micoletzkyi* Steiner, 1941

Family: ANOMYCTIDAE fam. nov.

Definition: One large (dorsal) and two small (subventral) oesophageal glands lying separately, dorsally in body and joining the alimentary canal behind the

median bulb. Head somewhat winged, with a shallow, sclerotized, saucer-shaped, frontal disc. Spear with long extension.

Genus: *Anomyctus* Allen, 1940

See above.

Type species: *Anomyctus xenurus* Allen, 1940

No other species.

Family: SPHAERULARIIDAE (Lubbock, 1861) Skarbilovich, 1947
syn. Family: Sphaerulariacea Lubbock, 1861

Definition: Median bulb absent but orifice of dorsal oesophageal gland just anterior to subventrals in the position typical for Aphelenchoidea. Parasitic female having vulva surrounded by perivaginal cells or more or less prolapsed. Parasites of insects.
Subfamily: Sphaerulariinae (Lubbock, 1861) Pereira, 1947 becomes redundant.

Genus: *Sphaerularia* Dufour, 1837

Definition: Spear without knobs. Tails short, conical to a point. Vulva very posterior; short post-vulval sac present. Male with spicules, more tylenchoid than aphelenchoid; gubernaculum present but bursa absent. Males and juvenile females escape from their host, after its death, and are free-living in the soil. The fertilized female enters a new host and the uterus greatly enlarges and prolapses, ultimately becoming 15,000 to 20,000 times the volume of the original female body which remains as a minute appendage to the uterus. Parasites of *Bombus* spp. and *Vespa* sp.

Type species: *Sphaerularia bombi* Dufour, 1837.

No other species.

Two of the following species are Tylenchs and the third is most likely so. They should probably be placed in the genus *Sphaerulariopsis* Wachek, 1955 (Tylenchoidea). KHAN (1960) puts his two species into *Stictylus* following RUEHM's (1956) making *Sphaerulariopsis* a synonym of *Stictylus*.

S. dendroctoni Massey, 1956
S. hastata Khan, 1957
S. ungulacauda Khan, 1957

Genus: *Tripius* Chitwood, 1935

syn. *Asconema* Leuckart, 1886
nec. *Asconema* Schmidt, 1880 (spongé)
nec. *Askonema* Kent, 1870 (protozoon)
syn. *Atractonema* Leuckart, 1887
nec. *Atractonema* Stein, 1878 (protozoon)

Definition: Very little information of recent date available, particularly no data about the orifice of the dorsal oesophageal gland. Apparently similar to *Sphaerularia* but in the female the prolapsed uterus remains no bigger than the female body. Parasite of the gall-midge *Cecidomyia pini* Deg. but BOVIEN (1944) suggests that the midge is more probably *Dasyneura abietiperda* Hensch.

Type species: *Tripius gibbosus* (Leuckart, 1886) Chitwood, 1935
syn. *Asconema gibbosum* Leuckart, 1886
Atractonema gibbosum (Leuckart, 1886) Leuckart, 1887

No other species.

Genus: *Proatractonema* Bovien, 1944

Definition: Male with feeble spear, knobless. No bursa; spicules and gubernaculum tylenchoid. Female spear well developed, its base slightly swollen and posteriorly tripartite. Fertilisation occurs when the female is pre-adult. After the final moult the adult grows and a cluster of perivaginal or uterine cells protrude through the greatly distended vulva in a partial prolapse. Parasitic in larvae of *Sciara* sp.

Type species: *Proatractonema sciariae* Bovien, 1944

No other species.

Genus: *Scatonema* Bovien, 1932

The position of this genus in the Aphelenchoidea is somewhat doubtful being based on the fact that the orifice of the dorsal oesophageal gland does not open near the spear base. Bovien says that the dorsal oesophageal gland is absent. The presence of perivaginal cells suggest aphelenchoid affinities.

Definition: Male without oesophageal glands or stylet. Spicules and gubernaculum tylenchoid; a slight bursa present. Female with well-developed subventral oesophageal glands. Gonad slightly developed but in parasitic female greatly developed. A prominent group of perivaginal cells present. Succession of generations can occur in the larvae, pupa and images of *Scatopse fusipes* Meig. and a second and even third parasitic generation may occur within the mother nematode. Males and non-parsitic femles occur free in manure or in egg-clusters of *Scatopse*.

Type species: *Scatonema wülkeri* Bovien, 1932

No other species.

Species inquirendae

The following species seem to belong in the superfamily but the details in their description are inadequate for recognition by present day standards. They are here listed alphabetically regardless of the genus in which they have been placed.

Aphelenchus alderholdi Schwartz, 1912
Parasitaphelenchus autographi Fuchs, 1930
A. coffeae Noack, 1898
Parasitaphelenchus conjunctus Fuchs, 1930
Parasitaphelenchus curvidentis Fuchs, 1930
P. dubius Fuchs, 1930
Aphelenchoides elongatus Schuurmans Stekhoven, 1951
Aphelenchus erraticus v. Linstow, 1876
Aphelenchoides ferrandini Meyl, 1954
Aphelenchus gracilis (Cobb, 1888) Cobb, 1891
syn. *Tylenchus gracilis* Cobb, 1888
T. cobbi de Man, 1906
Aphelenchus hessei Rahm, 1924
syn. *Aphelenchoides hessei* (Rahm, 1924) Fil. & Sch. Stek., 1941
Aphelenchoides hunti Steiner, 1935
Aphelenchus kongoensis Allgén, 1933
Parasitaphelenchus ligniperdae Fuchs, 1930
Aphelenchus littoralis Hofmänner, 1915
A. microlaimus Cobb, 1891
Aphelenchoides minimus Meyl, 1953
Aphelenchus minor Cobb, 1893
A. modestus de Man, 1876
A. mycogenes Schwartz, 1912
Aphelenchus naticochensis Steiner, 1920
A. ormerodis Ritzema Bos, 1891
Parasitaphelenchus pissodis notati Fuchs, 1930
P. pissodis piceae Fuchs, 1930
Aphelenchus pyri Bastian, 1865
A. retusus Cobb, 1927
syn. *Aphelenchoides retusus* (Cobb, 1927) T. Goodey, 1951
Parasitaphelenchus rhenanus Fuchs, 1930
Aphelenchus richtersi Steiner, 1914
A. rivalis Bütschli, 1873
A. striatus Steiner, 1914
A. villosus Bastian, 1865
Aphelenchoides xylophilus Steiner & Buhrer, 1934

The consequence of declaring *Aphelenchus ormerodis* a *species inquirenda* is to invalidate the subgenus *Chitinoaphelenchus* made by MICOLETZKY in 1922, of which *A. ormerodis* was the type (CHITWOOD, 1935). The recent promotion of *Chitinoaphelenchus* to generic rank by CHITWOOD in CORBETT (1959) to contain *Aphelenchus cocophilus* Cobb, 1919 is meaningless because it takes no account of Chitwood's action in 1935.

ZUSAMMENFASSUNG

Die Klassifizierung der Aphelenchoidea Fuchs, 1937

Es wird erwägt, die Aphelenchoidea wie folgendes zu verfassen: Aphelenchidae - *Aphelenchus*. Aphelenchoididae - *Aphelenchoides, Seinura, Megadorus, Tylaphelenchus, Laimaphelenchus, Bursaphelenchus, Rhadinaphelenchus, Cryptaphelenchus, Cryptaphelenchoides, Ektaphelenchus, Parasitaphelenchus, Peraphelenchus* & *Entaphelenchus*. Paraphelenchidae - *Paraphelenchus* & *Metaphelenchus*. Anomyctidae - *Anomyctus*. Sphaerulariidae - *Sphaerularia, Tripius, Proatractonema* & *Scatonema*.

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A REDESCRIPTION OF *XIPHINEMA DIVERSICAUDATUM* (MICOLETZKY, 1923 & 1927) THORNE, 1939 AND OBSERVATIONS ON ITS LARVAL STAGES

BY

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In 1958 HEWITT, RASKI & GOHEEN demonstrated for the first time the transmission of a virus by a nematode, namely fan leaf virus of grape vines by *Xiphinema index* Thorne & Allen, 1950. One of us (R.S.P.) found, early in 1958, specimens of *Xiphinema* in soil around the roots of strawberry plants infected with *Arabis* mosaic virus at Sutton Valence, Kent (ANON, 1959; JHA & POSNETTE, 1959) and submitted it to the first author (J.B.G.) for identification. Later another of us (F.C.P.) found a *Xiphinema* which he considered to be a new species associated with the roots of white clover at Crewkerne, Somerset (PEACOCK, 1959). Both lots of specimens were members of the same species and this appeared to be very close to *X. index* but differed in some morphological characters; both sexes were larger; males were common (about 40% of adult numbers) and had no innervated organ anterior to the ventral supplements; females had the vulva more posteriorly placed. The specimens appeared to belong to a new species, for the description and figures of *X. diversicaudatum* in THORNE (1939) did not tally with them. This view is based on the shape and size of the female tail (THORNE, 1939, fig. 145a) and the male tail (fig. 145b). However, the specimens did agree with the original descriptions of *X. diversicaudatum* female in MICOLETZKY (1923) and male in MICOLETZKY (1927). Figure 1 of the present paper shows outlines of the tails of both sexes as drawn by MICOLETZKY (1923 & 1927) and by THORNE (1939) together with drawings of the material studied. The magnifications have been adjusted to bring all drawings to a similar scale. Thorne's figures were magnified four times to bring them to the comparable scale, suggesting that his magnification (x 725) is twice too large. If this assumption is correct the tail pegs become (♀) 20 μ and (♂) 24 μ long (Table I), which is beyond the range of 130 specimens we measured. The comparison shows that Thorne's figures differ from those of Micoletzky and of our specimens; it suggests that neither male nor female depicted by Thorne is *X. diversicaudatum*: the shape of the female tail is like that of a larva (c.f. K).

In addition to the two sites already mentioned, the nematode has been found in

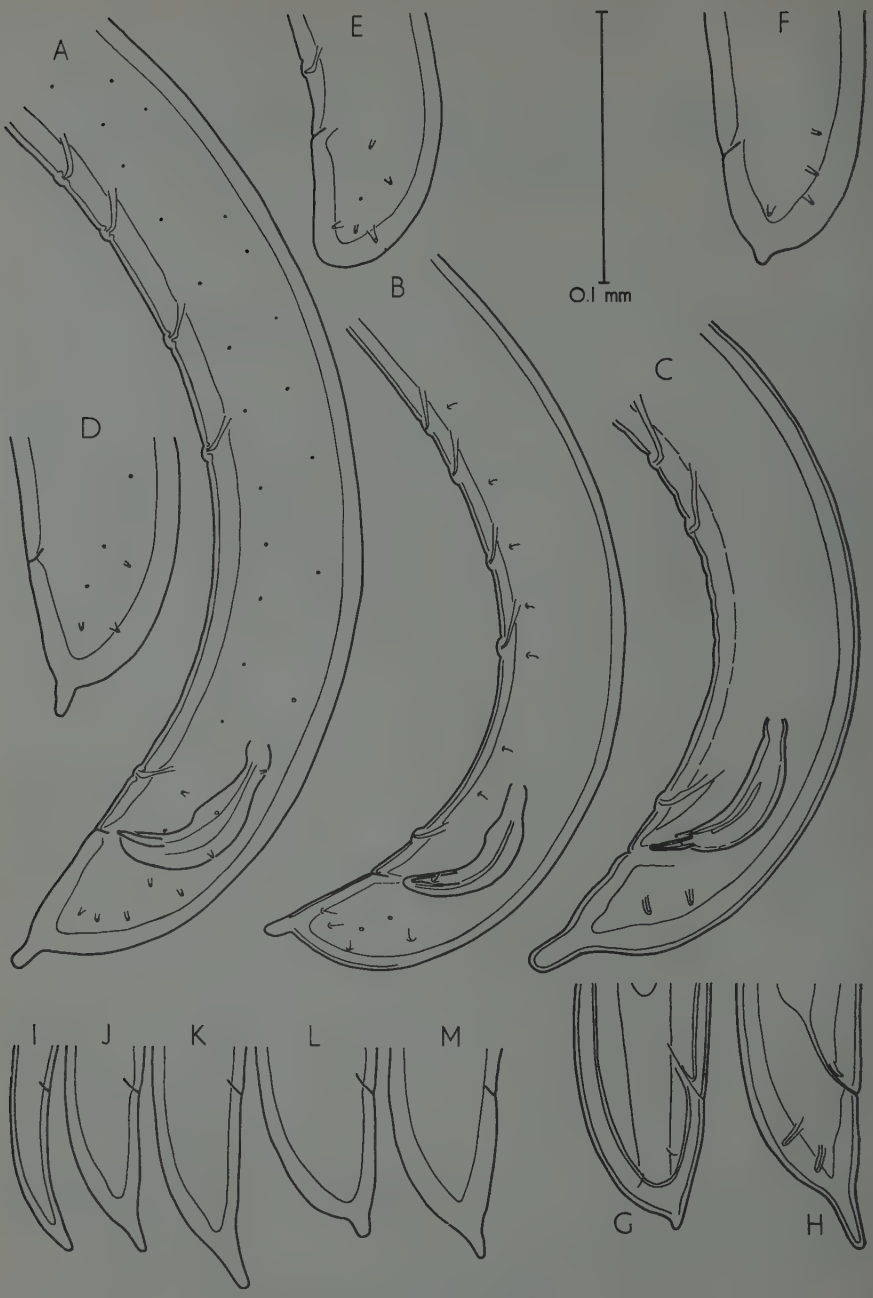

Fig. 1. Legend see next page.

all soils where the soil-borne, *Arabis* mosaic virus has been found (HARRISON & CADMAN, 1959); it occurred at Bere Ferrers, Bere Alston and Buckland Monachorum, Devon; Fareham, Hants.; Milsted, Doddington, and Elham, Kent; Methwold, Norfolk; Flatford Mill, Suffolk; Manorbier, Pembrokeshire.

Specimens derived from material with which SCHINDLER (1957) worked in U.S.A., and a male, female and larva from the Netherlands were also examined. All these agree with the specimens from Britain and are considered to be *X. diversicaudatum*.

As confusion seems to exist about the identity of this nematode, advantage was taken of the wealth of material to study its detailed morphology and its larval stages. A redescription of *X. diversicaudatum* follows.

XIPHINEMA DIVERSICAUDATUM (Fig. 1)

Table I shows measurements on several characters from the various populations we have examined and compares these measurements with those reported by MICOLETZKY (1923 and 1927) and THORNE (1939).

Cuticle thick with very faint criss-cross pattern occasionally visible. Anterior end narrow and lip region almost continuous with body. Lips fused and with an apical ring of six papillae, one on each lip and an outer ring of eight papillae, two per lip, but not on the laterals. Amphids stirrup shaped, as broad as the head base. On each side of the body, lateral fields visible as a hypodermal band beneath the cuticle; each begins very narrowly at the level of the front end of the posterior part of the oesophagus, then widens gradually, becoming about a quarter of body-width at mid-body-length. Through the cuticle above this band a row of pores is visible, beginning just behind the head and extending the full length of the body. This row of pores follows the dorso-lateral border of the band, but near the vulva and towards the tail end in both sexes the row tends to be double and follows each edge of the band. Dorsal and ventral pores are faintly visible near the head and sporadic ventral pores occur further down the body. Two to four pores occur laterally on the female tail and three to six on the male tail but the pattern on one side may be different from that on the other.

Spear long and similar in both sexes, about 143 μ, surrounded by a guiding sheath attached to the pharyngeal tube at about two-thirds the spear length, the base of the sheath appearing as a clear ring, averaging 128 μ from the anterior end. The length of this guiding sheath varies according to the position of the spear and sometimes when the spear is drawn right back the sheath appears to be folded back on itself below its fixed basal ring (Fig. 2). Spear extension about 83 μ

Fig. 1. *X. diversicaudatum*. A, ♂ tail end, showing spicules, lateral guiding pieces, arrangement of supplements and disposition of pores on the tail and along the edges of the lateral band; B, ♂ tail end, after Micoletzky, 1927; C, ♂ tail end, after Thorne, 1939; D, ♀ tail with long peg; E, pegless tail; F, short pegged ♀ tail; G, ♀ tail, after Micoletzky, 1923; H, ♀ tail, after Thorne, 1939; I-M, larval tails. I, L1; J, L2; K, L3; L, L4, preadult ♀; M, L4, preadult ♂; Magnifications all alike. N.B. The variation shown by the tails in A, D, E & F applied equally well to either sex.

TABLE I

Measurements on several characters from various populations of Xiphinema diversicaudatum

		Micoletzky 1923 ♀	Micoletzky 1927 ♂	British specimens ♀	British specimens ♂	U.S.A. specimens ♀	U.S.A. specimens ♂	Thorne 1936 ♀	Thorne 1936 ♂
n		1	2	43	33	5	6	?	?
Length	mm	4.0	4.2 (4.1-4.3)	4.9 (4-5.5)	4.9 (4.1-6.2)	4.2 (3.3-4.9)	4.2 (3.7-4.7	4.0	4.3
a		72	78 (74-81)	74 (57-92)	76 (57-96)	68 (54-80)	71 (59-80)	72	77
b		10	9.4 (8.6-10.1)	9.1 (6.6-11.4)	8.8 (7.4-11.3)	8.3 (6.9-9.4)	8.7 (7.7-10.3)	10.1	9.3
c		96	97 (93-103)	78 (61-134)	78 (55-100)	90 (54-120)	76 (68-98)	96	96
V or T %		?48[12]	49	15 43 15 (9.20) 39-46 (8.24)	58 (47-67)	17 45 18 15.19 43-49 16.20	58	9 48 10	49
Spear length	μ	133	130 (128-132)	143 (130-157)	143 (131-153)	131 (116-142)	128 (121-136)	130 [125]	
Spear extension	μ	78	?	85[a] (70-97)	83[b] (72-90)	86 (80-93)	83 (79-90)	65	
Guiding sheath length	μ	13	?	1.5-31[c]	3-25[d]	3-22	10-28		
Anterior end to fixed ring of guiding sheath	μ	?	c. 100	129[a] (115-143)	126[e] (119-134)	129 (114-140)	123 (95-141)	[91 or 96][h]	
Tail length	μ	48	44	52	56	50	53 (45-59)	[30]	[29]
Anal body width	μ	44	64	50	50	47	45	[21]	[23]
Widest cuticle on tail	μ	8.6	7.4	7-14	7-14	8-11	5-9		
Length of terminal peg	μ	3.6	10	9[f] (0-16)	10[g] (0-16)	7.5 (6-10)	11 (8-14)	[10]	[12]
Number of lateral pores on tail		2	6	2-4	3-6	2-4	3-6	2	2
No. of subventral pores			7-8		8-14		6-12		
Spicule length	μ		67		76 (69-81)		72.3 (72-74)		[34]
Lateral guiding pieces	μ		17.3		18 (16.5-20.7)		17 (14.5-21)		[9]
Number of ventral pores			3-4		3-5		3-4		3-4
Distance Cloaca-1st pair	μ		22		24		27		[12]
1st pair-1st single	μ		82		97		102		[57]
1-2	μ		32		38		39		[15]
2-3	μ		36		38		40		?
3-4	μ		20		38		33		?
4-5	μ				38				

Notes: [ ] data have been calculated from figures.
a, (n = 31); b, (n = 22); c, (n = 26); d, (n = 12); e, (n = 19); f, (n = 80); g, (n = 50); h, Thorne's figure 145 shows 2 basal rings
italics, these data appear to be half their correct value.

long, tripartite, with expanded, basal flanges. Anterior part of the oesophagus narrow but widening suddenly to form the basal bulb which forms just less than half the total oesophageal length. In the basal bulb the dorsal gland opens very near its anterior margin, the first pair of subventrals at about midway and the second pair about a half bulb-width from the base. The base of the bulb is flat with rounded edges, forming a butted junction with the intestine. The oesophago-intestinal cells form a small, low, rounded projection into the lumen of the intestine. About six cells forming the wall are visible in a section through the intestine. Pre-rectum is about twelve body-widths long, separated from the intestine by a constriction of the intestinal lumen. Rectum about two-thirds body-width long.

Female with median vulva, a transverse slit on the surface but vagina just below it becomes — + — shaped, and lower still the cavity is + shaped. Vagina extends

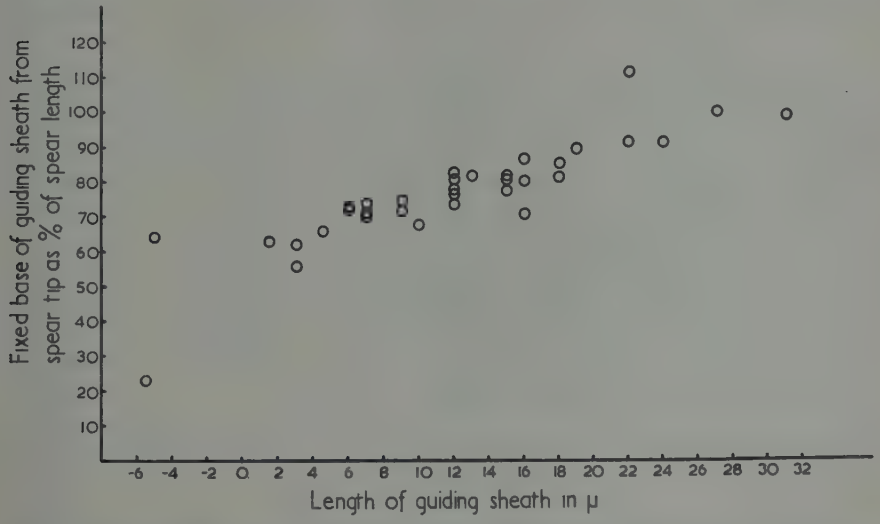

Fig. 2. The relationship between the length of guiding sheath and position of spear in *X. diversicaudatum*. N.B. The negative lengths indicate that the sheath was inverted with its free edge behind the fixed basal ring. The spear is well protruded from the mouth for the higher positive values.

just over half body-width and its walls are thick. A ventral view of the vulva shows four bands of muscles, arranged as a St. Andrew's cross (×), radiating from the central point. The female gonads are opposed and reflexed. Vagina opens into the junction of the two uteri where the organ more or less fills the body cavity. Each branch of the gonad is similar and consists of a uterus seven or eight body-widths long and often fairly convoluted, with, at its end distant from the vulva, a swollen part that may be a spermotheca. Following this is a narrower part formed of seven or eight rows of closely-packed cells round a narrow lumen. This may be comparable to the 'quadricolumella' described and named by Wu (1958) for *Ditylenchus destructor*. Beyond this is a wide, apparently glandular, part about

three body-widths long, practically filling the body cavity. A narrow oviduct of simple small cells about three or four body-widths long is joined to this glandular part by a sphincter valve which, in living specimens, often shows very clearly. SIDDIQI (1959) mentions this and figures one of apparently different form for *Xiphinema citri*. This sphincter valve appears to be widespread in the Dorylaimoidea judging by the examination of specimens from numerous genera. The oviduct connects directly with the largest cell of the ovary, the first of a reflexed, conically diminishing, row of cells about three body-widths long. The basic pattern of the gonads varies considerably. The uterus may contain eggs, averaging 195 x 44 μ (n = 12) and varying from four to seven times as long as wide. Up to four eggs may occur together, two in each uterus but one or two are the commonest numbers.

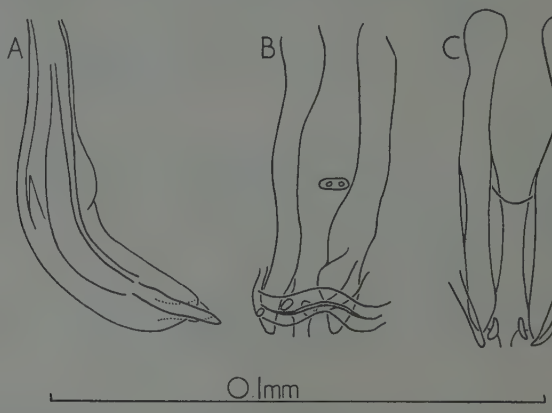

Fig. 3. The relationships of spicules, lateral guiding pieces and inner cuticularised pieces of ♂ *X. diversicaudatum*. A, lateral view; B, almost ventral view; paired preanal supplements showing; C, dorsal view.

The tail of both sexes is about an anal-body-width long, rounded, rather more so dorsally than ventrally, or more or less flattened ventrally with all the rounding on the dorsal side. A short, mammillate peg, rather variable in length, ends the tail more or less ventrally. The peg varied in 130 specimens from 0 to 16 μ in length with a mean of 9.6 μ. A few had no tail peg, and had there been no other characters to differentiate these pegless specimens from the others in the collection, they might well have been considered a separate species. Table II summarizes the incidence of these pegless forms encountered in two collections.

The male tail also has a pair of ventral supplements about a half-body-width pre-anal, then a gap of about two body-widths before a row of three to five ventral supplements spaced about four-fifths of a body-width apart. Just as the lateral pores on the tail are the posterior ending of the series of the lateral field pore system, so there is a series of pores on the ventral edge of the lateral field and associated with the prominent musculature of the hind end of the male body.

This row of latero-ventral pores varies from eight to fourteen with a mean of ten.

The spicules are large, averaging 76 μ along their axis. Laterally each appears to have a median band of thickening and from the dorsal or ventral aspect there

TABLE II

Numbers of X. diversicaudatum *from two populations, showing incidence of pegless tails* (—) *compared with those with pegged tails* (+)

Locality	♀		♂		Larvae pre ♀	Larvae pre ♂	Remainder including preadults
	+	—	+	—	—	—	+
Fareham	45	2	26	2	3	1	77
Flatford Mill	3		2		1		6

is an inwardly directed flange. Associated with the spicules is a pair of finger-like, lateral, guiding pieces about 18 μ long arising apparently from the walls of the spicular pouch. Between the spicules can be seen, in lateral view, a pair of shorter cuticularised pieces, about 11 μ long, with wider ends. Figure 3 shows the relationships of the spicules, lateral pieces and inner cuticularised pieces, from three aspects. It was not apparent to what these inner pieces were attached, but in dorsal view a faint transverse line could be seen about half-way along the spicules. This might be interpreted as the end of a gubernaculum but it more likely marks the junction of vas deferens and cloaca. The inner cuticularised pieces together with the lateral guiding pieces may be said to form a gubernacular system; the lateral guiding pieces probably should be regarded as crura (CHITWOOD & CHITWOOD, 1950, p. 121).

OBSERVATION ON LARVAL STAGES

The larva is recognisable by two spears, one in the normal position and a developing one in a cell situated in the ventral side of the anterior part of the oesophagus. When the last moult occurs the spear, its surrounding lining of the pharynx, the guiding sheath and the lining of the spear extension, are shed. Other moults were not observed but they are probably similar.

The new spear comes into position by piercing into the oesophageal lumen at its junction with the spear extension and travelling forwards to become attached to the anterior end of the spear extension. This appears to happen concurrently with the moulting of the old spear and it may well be that the separation of the lining of the spear extension at its base facilitates the passage of the new spear.

Each larval stage shows only the active spear and one developing, by contrast to some species of *Dorylaimus* in which a third stage larva, for instance, has two developing spears. After each moult a new spear develops in the oesophageal

TABLE III

Lengths of larval stages and of the spears they bear

Larval stage	n	Spear	Developing spear	Body length mm.
L1	1	56	73	1.13
L2	7	80	105	1.86
		(71-89)	(85-120)	(1.44-2.33)
L3	11	103	127	2.53
		(98-106)	(121-133)	(2.17-2.71)
L4	4	123	151	3.68
		(117-130)	(142-158)	(3.44-4.06)
L4 pre ♀	10	124	153	3.76
		(115-128)	(142-156)	(3.36-4.10)
L4 pre ♂	52	143		4.90
		(130-157)		(4-6.2)

cell and even in the adult there is a tiny spear tip to be seen enclosed in a clearer area. The spear lengthens during each moult and by measuring both spears the larval stage can be determined (Table III). Coupled with this difference in spear lengths are differences in tail shape, only the pre-adult larvae have tails similar to those of the adults. Earlier stages are relatively longer and narrower, without the offset peg. Fig. 1, I-M shows the different shapes characterising each of the stages.

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ZUSAMMENFASSUNG

Eine Neubeschreibung von Xiphinema diversicaudatum *(Micoletzky, 1923 & 1927) Thorne, 1939 und Beobachtungen über seine Larvenstadien*

Abbildungen und Beschreibung von *Xiphinema diversicaudatum* bei THORNE (1939) scheinen mit der Originalbeschreibung von MICOLETZKY (1923 & 1927) nicht übereinzustimmen. Die Art wird erneut nach Proben beschrieben, die mit der Originalbeschreibung sehr gut übereinstimmen. Beobachtungen werden mitgeteilt über die Variation der Schwanzform, der Zahl der kaudalen Papillen, der Zahl der subventralen Papillen beim Männchen, der Längenveränderung der Führungsscheiden mit der Stellung des Stachels und den Abweichungen, die sich aus den verschiedenen Larvenstadien ergeben.

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FURTHER OBSERVATIONS AND COMMENTS ON THE IDENTITY OF *ROTYLENCHUS ROBUSTUS* (DE MAN, 1876) FILIPJEV, 1934 WITH A DESCRIPTION OF A PROPOSED NEOTYPE AND A NEW DEFINITION OF *ROTYLENCHUS GOODEYI*

BY

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LOOF & OOSTENBRINK (1958) conclude that *Rotylenchus robustus* (de Man, 1876) Filipjev, 1934 is the same nematode as *Hoplolaimus uniformis* Thorne, 1949. We disagree with this conclusion, seek to show why it is wrong, and to establish a neotype.

The genus *Rotylenchus* was erected by FILIPJEV (1934a) with the type designated as *"Tylenchus robustus* de Man, 1880". Filipjev was aware of de Man's paper of 1876 because he cites it and used it as the basis to erect the Tylopharynginae. About the paper of 1880, Filipjev merely says "see 1884". We wonder whether Filipjev ever saw the paper of 1880: in it de Man gives a different set of dimensions for the female, gives dimensions for the male but calls the species *"Tylenchus robustus* dM" with the reference, just beneath, of „De Man, 1876 p. 125".

Later in the same year FILIPJEV (1934b) published the book *Nematodȳ vrednȳe i poleznȳe v sel'skom khozȳstve* [*Harmful und useful nematodes in rural economy*] in which he followed MICOLETZKY (1922) in placing *T. robustus* de Man, 1876 in *Tylenchorhynchus*, using for illustration (his figs. 119 & 120) Figs. 92, 92a and 92b of DE MAN (1884). LOOF & OOSTENBRINK (1958) point out that Figs. 92 and 92a do not tally with the 1884 description for the female and that they probably refer to some different species. We agree with this conclusion; Micoletzky may be correct in attributing to *Tylenchorhynchus* these figures but this can no longer be checked. The manuscript of Filipjev's book must have been written some months, perhaps a year or two, earlier, so that the views in his first paper (1934a) represent his later opinions. This throws some light on the choice of *T. robustus* de Man, 1880 as the type of *Rotylenchus* and indicates that Filipjev thought *T. robustus* de Man, 1876 to differ from *T. robustus* de Man, 1880.

Whatever the action by Filipjev in 1934 may have intended, the citation of *"Tylenchus robustus* de Man, 1880" must be examined in the light thrown on its meaning by the application of the *Règles Internationales de la Nomenclature Zoo-*

logique. It has generally been assumed that the citation means that nematode described by de Man in 1880, but this is wrong and not in accordance with the *Règles*. The report of the 12th Meeting of the International Commission on Zoological Nomenclature (*Bull. zool. Nomencl.* 1950, **4**, 177-180) makes it clear that the *Règles* deal with nomenclature and not with taxonomy. Article 30 of the *Règles,* on the designation of type species of genera, says:

"30 (a) When in the original publication of a genus, one of the species is definitely designated as type, this species shall be accepted as type, regardless of any other considerations".

In further clarification of certain ambiguities in Article 30 the Commission recommended

"that words should be inserted in the *Règles* to make it clear: (a) that Article 30 relates only to the designation, indication or selection of the type species of a nominal genus published subsequent to 31st December 1757, that is to say to the name of a genus as originally published subsequent to the above date by a given author in a given work, and that the action then taken by the author is alone relevant to the question: — (ii) of whether the type species of the genus in question is to be treated as having been designated under Rules (a), (b), (c) or (d) of the foregoing Article at the time of the original publication of the generic name concerned".

It follows, therefore, that the only possible meaning of the citation *"Tylenchus robustus* de Man, 1880" is the species *Tylenchus robustus* de Man, 1876 as used by de Man in 1880, because nomenclaturally the interpretation of the name depends exclusively upon de Man's action about it in 1876. The type, then, of the genus *Rotylenchus* Filipjev, 1934 is *Tylenchus robustus* de Man, 1876.

Filipjev amplified the data for the genus *Rotylenchus* by publishing twice in 1936; in a Russian journal (1936a) and in *Proc. helm. Soc. Wash.* where (1936b), the binomial *"Rotylenchus robustus* (de Man, 1880) n. comb." is used for the first time. In both publications the oesophagus is said to be aphelenchoid, ovaries double, head chitinized, cuticle coarsely striated and spear strong; in the latter paper he states that the spear is not very long, tail varying in size, bursa always caudal. As THORNE (1949) has pointed out, the term "aphelenchoid" applied to the oesophagus was intended to convey the idea that the oesophageal glands overlapped the intestine, and this is confirmed by the publication, in both papers, of the figure of the female from T. GOODEY (1932). Thus, the concept of the genus *Rotylenchus* requires a nematode with the oesophageal glands overlapping the intestine at the oesophageal-intestinal junction.

The original description (1876) (Fig. 1) by de Man of *Tylenchus robustus* gives the following data: The nematode lives rather commonly on the roots of plants growing in the moist ground of clayey meadows near Leiden. The spear is particularly strong and its length, as calculated from the text is ($^1/_5 \times {}^1/_6 \times$ 1.1 mm) = 36.7 μ, or from the given measurement data (10 ÷ 225) = 39.2 μ. The head is lightly coloured in transmitted light, is set off by a narrowing of the

body and has a short, arched ridge running posteriorly from the junction of the head and body (this is presumably the backward prolongation, for a few [5, 6 or 9 are figured] annules, of the basal plate). The lateral walls of the head show, on their upper surface, two curved, more or less parallel, cuticular "combs", which are presumably the ribs and spear guide of the head framework (though the inter-

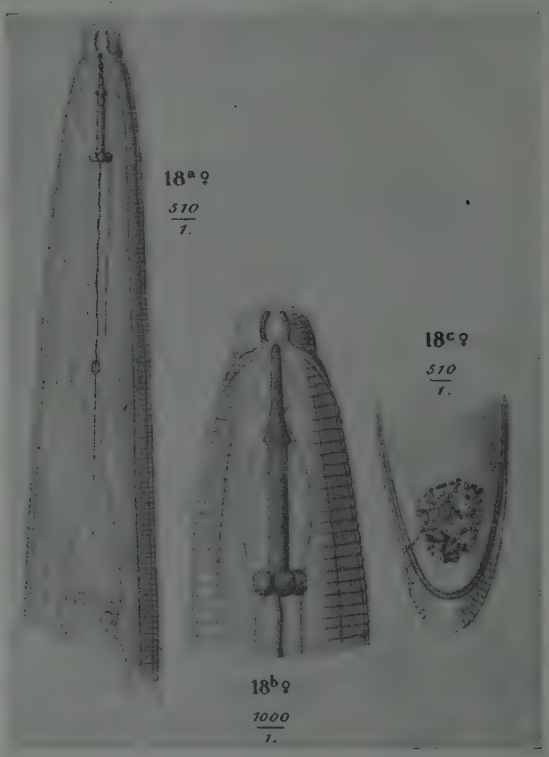

Fig. 1. Reproduction of plate 6 fig. 18a, b, and c of DE MAN (1876),
Tylenchus robustus de Man (1876) female, 18a: anterior portion, 510 x, 18b: head, 1000 x, 18c: tail 510 x.

pretation of the term "cuticulairkammen" [Cuticulärleisten in 1880] must probably remain ambiguous). The oesophagus has a procorpus with a recognisable lumen, an ovoid median bulb, somewhat difficult to see, and a wider spathulate, terminal part, depicted as being constricted where it joins the intestine but with its lumen visible only in the anterior part. It seems reasonable to interpret this configuration as indicating where the oesophageal lumen ends and the intestine begins, i.e. the oesophagus overlaps the intestine. Two large basal nuclei are also depicted.

The intestine is filled with dark granules. The tail is short and stumpy, the cuticle at the terminus rather thick with 12-13 annules beyond the anus but with the end apparently free from annules extending round its tip. The vulva is at 58.7 % (165 ÷ 281), the gonads are said to be asymmetrical and three or four eggs were found in the uterus. The asymmetry of the gonads may be explained by their being possibly overlaid by the granular intestine and so being difficult to discern with accuracy.

In 1880 de Man gave a somewhat different description, new dimensions but no new figure. He said that the specimens were commonly found in sandy, dune fields. Table I gives size data from DE MAN (1876 & 1880), from THORNE (1949), from OOSTENBRINK (1954), and from specimens of *Hoplolaimus uniformis* from an English source. Data from DE MAN (1884) are not germane to the arguments and are not quoted.

LOOF & OOSTENBRINK (1958), after examining the original slides of de Man's collection, pointed out that there exist two slides, one bearing a female the other a male, collected in 1879 from sandy dune soil at Katwijk, Netherlands. These slides are labelled *Tylenchus robustus* in de Man's own handwriting. Loof & Oostenbrink show that the specimens appear to be *Hoplolaimus uniformis* Thorne, 1949, and their dimensions make it quite likely that they are the actual specimens on which the descriptions of 1880 are based. Having established this identity, Loof & Oostenbrink then point out that the question to be answered is whether the nematode described by de Man in 1876 is the same as, or different from, that described by him in 1880. If they are the same, then we have to attach the concept of *Hoplolaimus uniformis* to the name *Rotylenchus robustus,* but if they are different *H. uniformis* and *R. robustus* remain separate species. They go on, however, to state that, if they are two different species, the latter, i.e. *Hoplolaimus uniformis* would be the type species of *Rotylenchus*, whereas we have shown above that the earlier specimen *Tylenchus robustus* de Man, 1876 is the type species, whatever its identity may be.

Before dealing with the arguments and conclusions set forth by Loof & Oostenbrink, there are one or two observations to be made concerning de Man's methods and behaviour. MICOLETZKY (1917) pointed out that de Man usually chose the largest specimen for description and DE MAN (1876 p. 122) states that the spear was measured from the anterior end of the body, not the anterior end of the spear, to its base. This increases the real length of the spear by about 5 μ (actually about 6 μ according to de Man's drawings). Loof & Oostenbrink overlooked this point and suggest that de Man did not see the fine point of the spear so that his spear was too short. Loof & Oostenbrink set out their arguments as four main points, prefacing them by a statement that:

"The description of 1876 is not very complete and has some puzzling statements which become clear, however, if we assume that De Man had, at that time, more than one species including *Hoplolaimus uniformis* synonomized and labelled as *Tylenchus robustus*".

TABLE I

Comparison of data for Rotylenchus robustus *and* Hoplolaimus uniformis *from various sources*

	Rotylenchus robustus					*Hoplolaimus uniformis*			
	De Man 1876	De Man 1880		Thorne 1949		Oostenbrink 1954		English population (n = 10)	
	♀	♀	♂	♀	♂	♀	♂	♀	♂
L	1.1 mm.	1.7	1.3	1.4	1.4	1.17-1.62	1.05-1.42 [1]	1.49-1.81	1.21-1.38
a	25.6	40	35	31	38	26	28	28-39	31-38
b	6.25	8	5-6	7.1	7.5	6.3	5.5	6.8-8.9	6.1-7.5
c	46.8	50-60	40	80-120	49	55	42	54-83	35-48
V	58.7%	postmedian		54%		55%		50.6-61.1%	
Spear from anterior end	39.2 or 37.7 μ								
Actual spear length	34.2 or 32.7 μ	42.5-53 μ		47 μ		50 μ (44) [2]	45 μ (34) [2]	39.6-47.5 μ	
Tail tip annules	Absent			Present		Present		Present	

[1] Misquoted by Loof & Oostenbrink as 1.26 i.e. the mean.
[2] Calculated from figures.

This basic assumption colours the conclusions derived from their arguments.

1. They point out that the species of 1876 is rather common in [clayey] meadows, whereas *H. uniformis* is decidedly not a meadow dweller.

2. De Man (1876) gives the length of *T. robustus* as 1.1 mm. and Loof & Oostenbrink point out that this is the maximal body length that has been considered, that it is small for *H. uniformis* [in fact it is outside the range of body length for all populations measured], and that, had he described *H. uniformis,* he would surely have found larger specimens. Yet they conclude that he must have seen a few *H. uniformis,* perhaps only one. This conclusion does not appear tenable.

3. De Man had an example with 3-4 eggs in the uterus. Loof & Oostenbrink point out that this is not normal for *H. uniformis* and that, in 1880, in what is undoubtedly *H. uniformis,* De Man found a single egg in the uterus. Loof & Oostenbrink conclude that the 1876 specimen, which had 3-4 eggs in the uterus, was perhaps a female of another species, not *H. uniformis.*

4. De Man found only females in 1876, whereas males of *H. uniformis* are not uncommon.

Having set forth these points, which seem to point away from, not towards, the identity of *Tylenchus robustus* de Man, 1876 as *Hoplolaimus uniformis,* Loof & Oostenbrink say that the colour and form of the lip region and the length of the spear are indicative of *H. uniformis.* The lip region of *H. uniformis* does certainly appear light or golden yellow in colour, but so does the lip region of other rotylenchs that are not *H. uniformis.* Likewise the arguments relating to spear length are not tenable. Reference to Table I shows that the actual spear length of the female of *H. uniformis* varies between 39.6 and 53 μ. The spear length as calculated from De Man's data is 36.7 or 39.2 μ, measured from the anterior end of the head to the spear base. It is thus about 5 μ longer than the actual length and when corrected measures, at most, 34.2 μ. This length is outside the range of spear length for *H. uniformis.*

Loof & Oostenbrink also say that the figure of De Man, 1876 (plate 6, fig. 18) shows Thorne's species but the opposite view is equally tenable.

Loof & Oostenbrink examined hundreds of samples from fields and allotments in the neigbourhood of Leiden. They found *H. uniformis* was very common in the sandy and medium textured soils but that it was rare in clayey fields. Of twenty-two more samples from meadows, *H. uniformis* was found in only one but never in an old meadow.

There appears, therefore, no valid evidence that *Tylenchus robustus* de Man, 1876 is identical with *Hoplolaimus uniformis,* and its identity has, if possible, to be determined. We suggest the following.

Collections by one of us (J.W.S.) of nematodes from clay soil near Leiden contained four females of *Rotylenchus* which appear to us to fit reasonably closely to the original description, by De Man in 1876, of *Tylenchus robustus.* One of these is hereby designated a neotype. Other specimens have been found at Wageningen, Zalt Bommel and Kapelle in clay soil and at Akersloot in sandy soil.

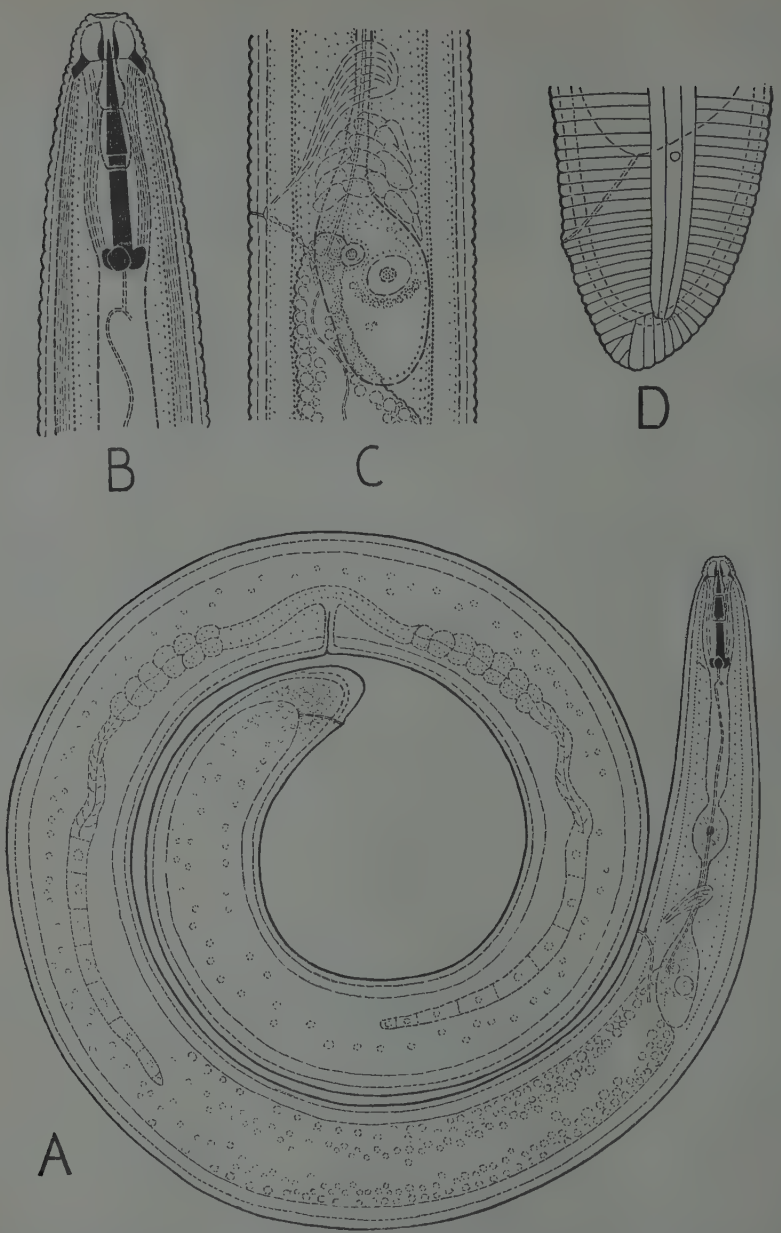

Fig. 2. *Rotylenchus robustus* (de Man 1876) Filipjev, 1934, female A: head 400 x, B: head 1000 x, C: region of junction of esophagus and intestine, 1000 x, D: tail 1000 x.

Neotype ♀ L = 1.06 mm, a = 23; b = 7; c = 52; V = $^{22}57.5^{22}$; spear = 34 μ.

Three other specimens from same locality:

♀ L = 0.93 — 1.2 mm, a = 22-23; b = 6.3-8.2; c = 45-60; V = $^{22\text{-}23}55\text{-}57^{18\text{-}22}$; spear = 35-36.5 μ

Specimens from other localities:

♀ L = 0.86 to 1.12 mm, a = 21-26; b = 5.6-7.5; c = 48-67; V = 54-58; spear = 33-37 μ

De Man's data:

♀ L = 1.1 mm, a = 25.6; b = 6.25; c = 46.8; V = 58.7; spear = 34.2 or 32.7 μ

Body relatively plump and tapering at both ends; forming a tight spiral when nematode is killed by gentle heat (Fig. 2A). Cuticular annules about 2 μ wide, distinct; lateral fields with 4 incisures, the outer ones crenate, occupying 1/4 to 1/5 of body-width, beginning as two rows of areolae at about 7 annules from lip base and becoming three bands just behind level of spear knobs; outer bands areolated till level of median bulb; ending just short of tail tip as in Fig. 2D. Phasmids small, about 8 annules (1/2 anal-body-width) anterior to anus. Head narrower than body, offset, hemispherical with 7 annules. Labial framework strongly cuticularised, yellowish by transmitted light, its outer margin extending posteriorly for about 2 body annules. Spear with heavy knobs that are flattened anteriorly (Fig. 2B). Orifice of dorsal oesophageal gland 7 μ from base of spear (20% of spear length). Procorpus of oesophagus cylindrical, narrowing slightly before expanding to join the small, medium-sized, oval, median bulb; isthmus narrower than procorpus but about the same length and where it joins the narrow anterior end of the intestine an oesophago-intestinal valve is present. Oesophageal glands contained in an oval body, rather variable in shape, but, when well developed, about a body-width long, overlapping dorso-laterally the junction of oesophagus and intestine. One gland nucleus easily visible, a second obscure but the third not observed in any specimens. The two nuclei are usually found in the anterior part of the glandular diverticulum. The posterior part, when well developed, often does not contain a nucleus but in a few instances, however, where there was apparently one present in the posterior part the anterior part contained only a single nucleus. Excretory pore at level of oesophago-intestinal junction. Hemizonid similarly placed. Intestine marked by large-sized granules. Rectum about 3/4 anal-body-width long. Vulva slightly post-equatorial; gonads paired, opposed, outstretched; oocytes in single rows; no spermathecae. Tail short, more or less conical and rounded, the tip toward the ventral side, annulations continuous round tip; cuticle of terminus thicker than on rest of body, between 1/3 and 1/2 tail-length.

Neotype: female collected from clay soil overgrown with grass at Hoogmade near Leiden, Netherlands. [Type locality is moist ground of clayey meadows near Leiden.] Slide no. T.C. 10/1 in I.P.O. nematode collection, Wageningen, Netherlands.

Male unknown.

Discussion. The measurements quoted above, for the neotype, De Man's original specimen, and the other recently collected specimens show reasonably close agreement; in particular the spear lengths agree well. Other points are compared and commented upon in Table II, where the points of similarity outweigh the differences. The differences can be attributed to the lack of detail in the 1876 data and to the ambiguities in De Man's description when considered with the same rigour applied to contemporary descriptions.

The designations by Loof & Oostenbrink (1958) of De Man's specimens as Metatype (slide no. 208 ♀) and Allolectotype (slide no. 209 ♂) can no longer stand.

In redefining *Rotylenchus robustus* = *Hoplolaimus uniformis,* Loof & Oostenbrink (1958) placed *Anguillulina robusta* ♂ of T. Goodey, 1932, *R. robustus* ♂ of Filipjev & Schuurmans Stekhoven, 1941 and *R. robustus* of Andrassy, 1952 as synonyms. The male tail, Fig. 61 of T. Goodey, 1932 is a copy of De Man's Fig. 92b of 1884 and Filipjev & Schuurmans Stekhoven copied Goodey; this figure may well have been drawn from preparation no. 209 of De Man's collection which Loof & Oostenbrink examined and concluded was *H. uniformis.* They showed that it was probably the basis for the 1880 description of the male which was not accompanied by a figure; the first time a male was figured was 1884. We agree, therefore, with Loof & Oostenbrink that the male of de Man 1884 is probably *H. uniformis.*

Andrassy, 1952, gives L = 1.114 mm., a = 24.8; c = 63.3; V = 56.8 %; 6-7 head annules; phasmids pre-anal; lateral field occupying 0.23 of body-width with 4 incisures; nothing is said about spermathecae; nor is the orifice of the dorsal oesophageal gland figured. On these data, for a single female and three larvae, it seems impossible to determine the species.

Loof & Oostenbrink (1958) also coined a new name, *Rotylenchus goodeyi,* to accommodate *Anguillulina robusta* ♀ of T. Goodey, 1932, adding as synonyms, the following: —

A. robusta of T. Goodey, 1940
R. robustus ♀ of Fil. & Sch. Stek., 1941
R. robustus of Thorne, 1949
R. robustus of T. Goodey, 1951
R. robustus of Golden, 1956

This synonomy is too inclusive because some species have spermathecae, others not. *A. robusta* of T. Goodey, 1932 is figured without spermathecae and reference to the original specimen confirms this; we consider this nematode to be *R. robustus*

TABLE II

Comparison of various characters of R. robustus *in original of de Man, 1876 and in Neotype*

	de Man. 1876	Neotype	Comments
Head shape	Hemispherical	Hemispherical	
Head annules	9	7	
Ratio spear parts anterior: posterior	1 : 1.6	1 : 1 1	
Peripheral backward extension of head framework extending through	5, 6 or 9 annules	2 annules	Clear that original specimen had this structure but seems possible that de Man was hazy on this point; his drawing is obviously ambiguous.
Median bulb shape ratio width: length	c. 1 : 1.4	c. 1 : 1.4	
Oesophageal gland nuclei visible	2	2	
Junction of oesophagus and intestine	Details obscure	Dorsolateral overlap of glands	De Man figures the oesophageal lumen as disappearing before the constriction that marks the differentiation of oesophagus from intestine. Seems reasonable to interpret this as an overlap of oesophagus and intestine. There is no controversy on this point.
Spear knobs	rounded	anteriorly flattened	De Man's drawings do not appear to reach the accuracy we are accustomed to expect today. Reference to many of his figures of spears of Tylenchs in his 1884 paper make this quite clear. The difference here noted is probably more apparent than real.
Annules, or equivalent, beyond anus i.e. tail annules	10 + (3) + (4) = 17	13	
Tail tip	without annules	annulated	De Man depicts the tail tip without annules whereas the neotype is annulated. Both, however, have the much thicker cuticle at the terminus. It can be postulated that de Man's specimen was not truly lateral, for in that position the annules can be obscured, but we have not evidence for this hypothesis.

as redefined above. The figure 121a in Filipjev & Schuurmans Stekhoven, 1941 is a copy of T. Goodey's 1932 figure.

THORNE's (1949) figures fit the conception of *R. robustus* fairly well for body measurements, spear length and absence of spermathecae but the head has only 4 or 5 annules and there are three oesophageal gland nuclei shown. On balance we consider that this nematode is not *R. robustus* but say nothing more about its position. The meagre data of GOLDEN (1956) give the spear as about 28 μ and the phasmids occurring at about anal level. This is quite insufficient for an identification so the synonomy of Loof & Oostenbrink that *R. robustus* of Golden, 1956 = *R. goodeyi* falls.

A. robusta of T. Goodey, 1940 and *R. robustus* of T. Goodey, 1951 both have spermathecae and thus obviously differ from *A. robusta* of T. Goodey, 1932 = true *R. robustus*. Figs. 23 and 24 of T. Goodey, 1940 show specimens with non-overlapping junctions of oesophagus and intestine and with 6 or 7 head annules, but Fig. 25 shows a head with 4 annules. Only the male specimen from which these figures were drawn still exists; it shows a lateral overlap of oesophagus and intestine and its head has 3 annules. T. Goodey clearly erred in the number of head annules, another instance of this can be seen in Fig. 51c of T. Goodey, 1951 where the head of *Pratylenchus pratensis* has seven annules. *P. pratensis* never has more than three or four annules and, in fact, no known species of *Pratylenchus* has more than four. Several other female specimens, which we consider to be the same species as *A. robusta* of T. Goodey, 1940 also have three head annules. Evidence from the original drawings shows that Fig. 50a in T. Goodey, 1951 is based on Fig. 23 of T. Goodey, 1940 and that T. Goodey corrected his interpretation of the oesophago-intestinal junction when he showed the overlap in 1951. Figs. 50c & d (T. GOODEY, 1951) are copies of Figs 27 & 30 of T. Goodey, 1940 and their originals show that they are the tails of the whole nematodes shown in Figs. 23 & 24 of T. Goodey, 1940. The head, with 4 annules, and oesophageal region in Fig. 50b of T. Goodey, 1951 was drawn from a specimen that no longer exists, Fig. 25 of T. Goodey, 1940 is the anterior part from the same original drawing.

In the list of synonyms of *R. goodeyi,* Loof & Oostenbrink put *A. robusta* of T. Goodey, 1940 first and to this the conception of *R. goodeyi* must now be attached. Bearing in mind the errors in Figs. 23 & 24 of T. Goodey, 1940 and that the 1951 Figs. 50a, c & d are based on the same specimens, the following is a short description to give stability to this species but more work needs to be done to establish its range of variation.

Rotylenchus goodeyi Loof & Oostenbrink, 1958

Figs. 50a, c & d in T. Goodey, 1951

Data taken from original drawings or calculated from published figures.

♀ (1940) L = 0.99 mm, a = 28; b = 6.6; c = 53; V = $^{23}61^{22}$; spear = 35 μ; spear base to orifice of dorsal oesophageal gland = 7 μ

♀ (1951) L = 0.98 mm, a = 27; b = 6.2; c = 53; V = $^{20}60^{20}$; spear = 33 μ; spear base to orifice of dorsal oesophageal gland = 7 μ

♂ (1940) L = 0.75 & 0.83 mm, a = 27 & 29; b = 6; c = 26 & 31; spear 27 & 28 μ; spear base to orifice of dorsal oesophageal gland = 6 μ; spicules 27 & 30 μ; gubernaculum = 12 & 14 μ

♂ (1951) no longer possible to tell where the data in T. Goodey, 1951 originated.

Body tapering anteriorly; head somewhat offset, relatively high and rounded, with 3 annules; basal plate of head framework continued laterally and posteriorly for 2-3 body annules. *Female*: spear about 34 μ long, basal knobs anteriorly concave, orifice of dorsal oesophageal gland about 7 μ from spear base. Vulva post-equatorial; gonads paired, opposed, outstretched; spermathecae present between ovaries and uteri. Tail about an anal-body-width long, broadly rounded. 4 incisures on body, occupying 1/4 body-width, not continuing round tail tip. *Male*: spear shorter, 27-28 μ long shaped like that of female. Tail short, pointed; just more than an anal-body-width long, ventrally curved; spicules 27-28 μ long, curved ventrally, proximally knobed. Gubernaculum curved anterio-ventrally, distally reflexed, 12-13 μ long. Bursa enveloping tail.

The standing of the species reviewed in the foregoing paper can be summed up as follows: —

Rotylenchus robustus (de Man, 1876) Filipjev, 1936
syn. *Tylenchus robustus* de Man, 1876
Anguillulina robusta ♀ of T. Goodey, 1932
R. robustus ♀ of Fil. & Sch. Stek., 1941

Rotylenchus goodeyi Loof & Oostenbrink, 1958
syn. *Anguillulina robusta* of T. Goodey, 1940 emended
R. robustus of T. Goodey, 1951 (partim)

Hoplolaimus uniformis Thorne, 1949
syn. *Tylenchus robustus* of de Man, 1880, 1884, ? 1917

Status inquirendae:
Rotylenchus robustus of Thorne, 1949
R. robustus of Andrassy, 1952
R. robustus of Golden, 1956

Acknowledgments. We are grateful for advice from Dr. Richard Mellville, Assistant Secretary to the International Commission on Zoological Nomenclature and from Dr. Ellsworth Dougherty, a member of the Commission.

ZUSAMMENFASSUNG

Weitere Wahrnehmungen und Beobachtungen über die Identität von Rotylenchus robustus *(de Man, 1876) Filipjev, 1934 mit einer Beschreibung eines vorgeschlagenen Neotyps und eine neue Definition von* Rotylenchus goodeyi

Die Autoren sind nicht mit der Auffassung von Loof & Oostenbrink (1958) einverstanden dass

Rotylenchus robustus (De Man 1876) und *Hoplolaimus uniformis* Thorne 1949 eine und dieselbe Art darstellen.

Nach den Internationalen Zoologischen Nomenclaturregeln ist *Tylenchus robustus* De Man 1876 der Typus des Geschlechtes *Rotylenchus* Filipjev 1934, denn Filipjev's Zitat *Tylenchus robustus* De Man 1880 kann keine andere nomenclatorische Bedeutung haben als *T. robustus* De Man 1876.

In verschiedener Hinsicht (Körperlänge, Länge des Stachels, Ausbildung der Schwanzspitze, Fundort) ist *T. robustus* nach der Beschreibung von De Man (1876) so verschieden von *Hoplolaimus uniformis*, dass sie kaum dieselbe Art darstellen können.

In der Gegend des typischen Fundortes von *Tylenchus robustus* De Man 1876 (Wiesen auf tonigem Boden in der Nähe von Leiden) wurden einige Tiere gefunden, die in fast allen Einzelheiten (u.a. Körperlänge, Länge des Stachels und Form der Schwanzspitze) sehr gut mit De Man's Beschreibung von 1876 übereinstimmen. Eins dieser Tiere wurde als Neotyp von *Rotylenchus robustus* (De Man, 1876) gewählt.

Anguillulina robusta von T. Goodey 1932 und *Rotylenchus robustus* (De Man, 1876) sind dieselbe Art. *Anguillulina robusta* von T. Goodey 1940 und 1951 ist nicht identisch mit *Rotylenchus robustus* (De Man, 1876) und bleibt *Rotylenchus goodeyi* Loof und Oostenbrink 1958.

R. robustus von Thorne, 1949 ist weder identisch mit *R. robustus* (De Man 1876) noch mit *R. goodeyi* Loof und Oostenbrink.

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Received for publication, April 28. 1960.

Nematologica 5 (1960): 149. E. J. Brill, Leiden

SHORT COMMUNICATIONS

M. T. Hutchinson [1]) & H. T. Streu [2]), *Tardigrades attacking nematodes.*

In June, 1959, soil collected from around the roots of wild blueberry *Vaccinium* sp., near Toms River, New Jersey, was processed by a method derived from the Baermann funnel. The soil was spread on cheesecloth on the mesh of a wire basket on legs in a deep pan with water just covering the soil. In the extract numerous, unidentified Tardigrades were found attacking nematodes. among which were *Trichodorus aequalis* and *Tylenchus* sp. Distinct tears were made by the tardi-

Fig. 1. A tardigrade attacking a nematode, *Tylenchus* sp.

grade stylets in the cuticles of the nematodes, and attacked nematodes subsequently became immobilized. Tardigrades were often so firmly joined to the nematodes that some could be mounted in Berlese fluid and photographed under a phase contrast microscope (Fig. 1). A microscope slide in the nematology collection at the Tea Research Institute, Talawakele, Ceylon, also showed a tardigrade attacking a nematode, indicating that the observation in New Jersey was not an isolated case.

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[2]) Agriculture Experiment Station, Rutgers University, New Brunswick, New Jersey, U.S.A.

R. W. Timm.[1]): *The Widespread Occurrence of the Hemizonid.*

The mysterious hemizonid of J. B. Goodey (1951) was exposed by the same author (1959), at the suggestion of B. G. Chitwood, as a ventro-lateral commissure of the nervous system. Goodey (1959) observed nerve fibres between the hemizonid and the circumoesophageal commissure or nerve ring in *Dolichodorus heterocephalus*. I have since seen this connection in many plant-parasitic nematodes. Up to now, however, the hemizonid has been regarded as a structure confined to the Tylenchoidea. From a knowledge of the general structural plan of a nematode one might also expect to find a similar feature in free-living nematodes and even in animal-parasitic nematodes as well.

Timm (1956) first recorded and figured the hemizonid in a free-living nematode, *Pellioditis marina* var. *bengalensis* (Timm, 1956) n. comb. [= *Rhabditis* (*Choriorhabditis*) *marina* var. *bengalensis*], though without indicating any connection with the nerve ring. After examining a number of soil, marine, and small invertebrate-parasitic nematodes for the presence of a hemizonid and its connection with the nerve ring I now conclude that, although often obscure or in a diffuse form, such a structure is widely, if not universally, distributed in the phylum. Figure 1 shows a number

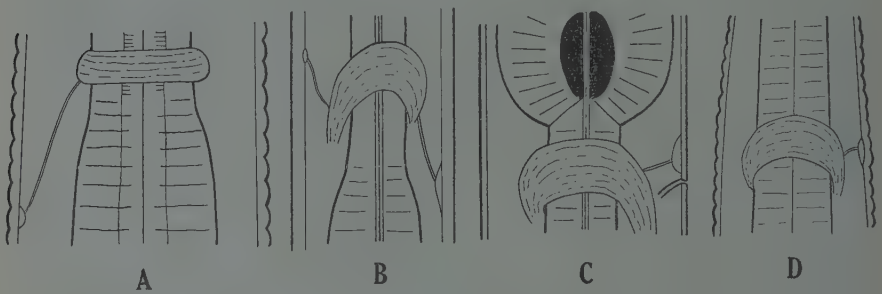

Fig. 1. Region of nerve ring, showing commissures and their connection with the nerve ring. A. *Leidynema appendiculatum*. B. *Dorylaimus* sp. C. *Eudiplogaster* sp. D. *Stephanolaimus* sp.

of examples from various habitats: *Leidynema appendiculatum*, a parasite from the hindgut of the cockroach *Blatta orientalis*; *Dorylaimus* sp., a soil dorylaimoid; *Eudiplogaster* sp., a soil rhabditoid; and *Stephanolaimus* sp., a marine form.

In some cases the ventro-lateral commissure is button-like and somewhat refractive, causing a bulging of the hypodermis and cuticle. In other instances the structure is flat and thin or non-refractive, and scarcely distinguishable. In still other cases no commissure can be seen at all, but there is present a fibrous connective, usually filamentous, from the nerve ring to the body wall. This connective is especially prominent in those species which have a spacious body cavity, *e.g.*, dorylamoids, in which a faint dorsal commissure can also sometimes be discerned (Fig. 1, B). Finally, in some species (or specimens of the same species) no commissure or connective can be observed at all.

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[1]) Notre Dame College, Dacca, East Pakistan.

BOOK REVIEWS

J. B. Goodey, Mary T. Franklin & D. J. Hooper. *Supplement to The nematode parasites of plants catalogued under their hosts, 1955-1958.* Commonw. agric. Bur., Farnham Royal, Bucks., 1959, 66 p.

The supplement comprises the new hosts of plant parasitic nematodes published from 1955 to 1958. It adds some 2500 records (extracted from 332 publications) to the 1956 main work, which means an increase of more than 50% in four years. A number of new records by the authors themselves and of "in litt." records are also included. For the arrangement of entries and references the same pattern has been used as in the main work (c.f. the review in *Nematologica* 1: 354). It is evident that the long host lists of some polyphagous root-infesting nematodes are to a large extent responsible for the large size of the supplement.

M. Oostenbrink

Mary T. Franklin & D. J. Hooper (1959). *Plants recorded as resistant to root-knot nematodes* (Meloidogyne *spp.*). Techn. Comm. Commonw. Bur. Helminth. 31 : 33 p.

The purpose of this compilation is to bring together the published records of plants showing complete or partial resistance to identified *Meloidogyne* species. In Section I about 310 resistant plant species and varieties are listed alphabetically, followed by the species of *Meloidogyne* to which they are resistant with the author and date of record. All known authors who have recorded a plant as resistant to a species are listed. In Section II the plant species are arranged in their families and listed under each of the seven *Meloidogyne* species under consideration. Inconsistant records are indicated. The compilation is drawn from 87 publications, listed in Section III.

M. Oostenbrink

PLANT PATHOLOGY, Problems and Progress 1908-1958. Edited by C. S. Holton, G. W. Fischer, R. W. Fulton, Helen Hart and S. E. A. Mc Callan. The University of Wisconsin Press, Madison 1959, XIX and 588 pages.

In this book the lectures given during the Golden Jubilee Meeting of the American Phytopathological Society held in 1958 at Bloomington Ind. U.S.A. are published. It consists of ten parts. Part I contains the Major Addresses on Historical and Developmental Aspects of Plant Pathology and Part II to X the nine symposia on: Physiology of Parasitism, Genetic Approach to Elucidation of Mechanisms Governing Pathogenicity and Disease Resistance, Fungicides, Chemistry of Fungicides, Soil Microbiology and Root Disease Fungi, VII Concepts and Problems of Nematology, Structure of Viruses, Multiplication of Plant Viruses and Epidemiology of Plant Diseases.

I shall limit my review to part VII. The reader will no doubt find extensive reviews of the other parts in phytopathological journals. Part VII (p. 383-434) deals with Concepts and Problems of Nematology in five chapters:

Historical highlights of nematology (by D. J. Raski), Ecological relationships of nematodes (by F. G. W. Jones), Variation in nematodes (by G. Thorne and M. W. Allen), Mechanism of nematode injury to plants (by J. R. Christie and V. G. Perry) and Progress in Chemical control (by A. L. Taylor), preceded by an Introduction (by J. N. Sasser). They are dedicated entirely to the fundamental aspects and problems of plant nematology and contain concise reviews of the present knowledge and basic concepts of the treated subjects. But it is perhaps a greater merit that the authors give ample attention to the problems still to be solved, try to indicate what gaps still exist in our knowledge and stress the importance of more fundamental research. As is unavoidable in this type of publication, probably not all generalisations made are as widely applicable as suggested by the text. So it is doubtful in my opinion, whether occasional development of a few nematodes on poor hosts often has much to do with genetic variation of the nematode species involved, as is thought by Thorne and Allen (p. 414). It seems more an expression of the fact that even a poor host still gives some opportunity to the nematode to live on it, perhaps only in few very small areas scattered over the roots or stems of the plant.

The opinion that most soil nematodes can withstand desiccation (Jones p. 402) is probably too inclusive. It is also doubtful whether in general the moisture characteristic curve supplies most of the necessary information regarding soil factors (p. 399).

Biotic factors other than food supply may be more important in determining the activity of nematodes in the soil and the relative abundance of certain species in certain soils. The extremely lacunar knowledge of ecological relationships of nematodes in the soil and of the influence of these on the distribution and abundance of nematodes could have received more attention. For a better understanding not only more research on nematode ecology is needed but also more information on population dynamics.

The chapters here reviewed should stimulate further research and, as Christie & Perry say in their excellent contribution, "should appeal to those who seek the challenge of the unpredictable."

J. W. SEINHORST

ANNOUNCEMENT

Fiftieth anniversary of the Helminthological Society of Washington.

On Saturday, October 8, 1960, the Helminthological Society of Washington will celebrate its Fiftieth Anniversary. It is hoped that all interested in parasitology and the related sciences will keep this date in mind and arrange to attend. The scientific program will be held at the University of Maryland, College Park, Maryland. A banquet will be held during the evening at the Naval Officers Club at Bethesda, Maryland. Dr. Chauncey D. Leake, President of the American Association for the Advancement of Science, will be speaker of the evening.

Nematologica 5 (1960): 153-157. E. J. Brill, Leiden

THE HATCHING RESPONSE OF *MELOIDOGYNE* SPECIES TO THE EMANATIONS FROM THE ROOTS OF GERMINATING TOMATOES

BY

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Larvae of *Meloidogyne* species emerge readily from egg masses when moisture, temperature, and aeration are favorable (GODFREY, 1931; BERGESON, 1959). In studying substances emanating from host plant roots that attract root-knot nematodes (VIGLIERCHIO & LOWNSBERY, 1957; LOWNSBERY & VIGLIERCHIO, 1958, 1960) the presence and extent of the hatching stimulation of such roots on egg masses of several *Meloidogyne* species was estimated. The tests were conducted throughout with very young seedlings of Rutgers tomato.

METHODS

The hatching effect was assessed by either, or both, of two methods.

Method I. Fresh egg masses, hand-picked for uniformity in size, color and opacity were centered in a layer of moist sand (1-2 mm thick) at the bottom of a Syracuse dish. A vigorous, three-day old seedling, selected for uniformity in length and straightness from a stock germinated at 25° C in the dark on moist filter paper, was placed 5 mm from the egg masses. After 24 hours in a dark, humidity cabinet at 25° C, the egg masses were removed and the sand placed on a Baermann funnel from which the larvae were collected and counted after 72 hours. Controls had no seedlings.

Method II. Small squares of gauze (38 mm × 38 mm) were cut so as to fit over an embryo dish. Water was added to the dish until the surface remained in contact with the sagging, wet square of gauze extending to the sides of the dish. Fresh egg masses placed in the center of the gauze would be wet but yet well exposed for aeration from above. Seedlings selected as in Method I were placed about the egg masses on the gauze so that the radicles protruded through the weave openings. Controls had no seedlings. Larvae were collected periodically by lifting a corner of the gauze, inserting a pipette and withdrawing the contents of the dish. The egg masses and dish were rinsed once and the washings withdrawn as before and combined with the initial suspension. In Method I the total sample in each replicate was counted while in Method II one aliquot from each replicate was counted after dilution.

RESULTS

The results of hatching tests using Method I are shown in Table I, and Method II in Table II.

TABLE I

Emergence of larvae (24 hr.) from egg masses of Meloidogyne *spp. with and without a single Rutgers tomato seedling*

Nematode	Seedling	Mean Number of Hatched Larvae	S.E.	Least Significant Difference 1% Level	Mean % Increase
M. hapla	+	77	4.8		
5 egg masses (48 replicates)	—	41	3.0	15	87
M. incognita acrita	+	146	8.2		
4 egg masses (60 replicates)	—	111	7.1	28	31

TABLE II

Hatching of larvae from egg masses of Meloidogyne *spp. in embryo dishes with and without Rutgers tomato seedlings. Each test consisted of 15-20 replicates with seedlings and 10-20 replicates of controls*

Nematode	Duration of Hatching	Number Hatched Seedlings	Mean of Larvae No Seedlings	Mean Difference	Least Sig. Diff. 1 % Level	Mean % Increase
M. hapla	0- 1 day	1,402	1,125	277	264	24.6
41 egg masses 15 seedlings	0-10 days*	17,750	14,820	2,930	1,520	19.8
M. javanica	0- 2 days	12,400	9,470	2,930	2,070	31.0
Aliquots of suspension of egg masses 15 seedlings	0-10 days*	38,700	29,500	9,200	5,330	31.2
M. incognita acrita	0- 1 day	1,120	954	166	128	17.4
37 egg masses 10 seedlings	0- 8 days**	13,800	12,100	1,700	1,630	14.0

* The hatched larvae were collected as described on the second, fourth, seventh and tenth days and distilled water was added to the dishes.

** The larvae were collected as described on the second, fifth and eighth days.

DISCUSSION

The results, with the systems used, indicate a significant increase in hatch of larvae due to the emanations from seedling roots. The response, demonstrated by two different methods, was not only detectable, but significant within 24 hours with *M. hapla* and *M. incognita acrita* and probably so with *M. javanica.*

The increase in the number of larvae hatching was of the order of 15-30 % for

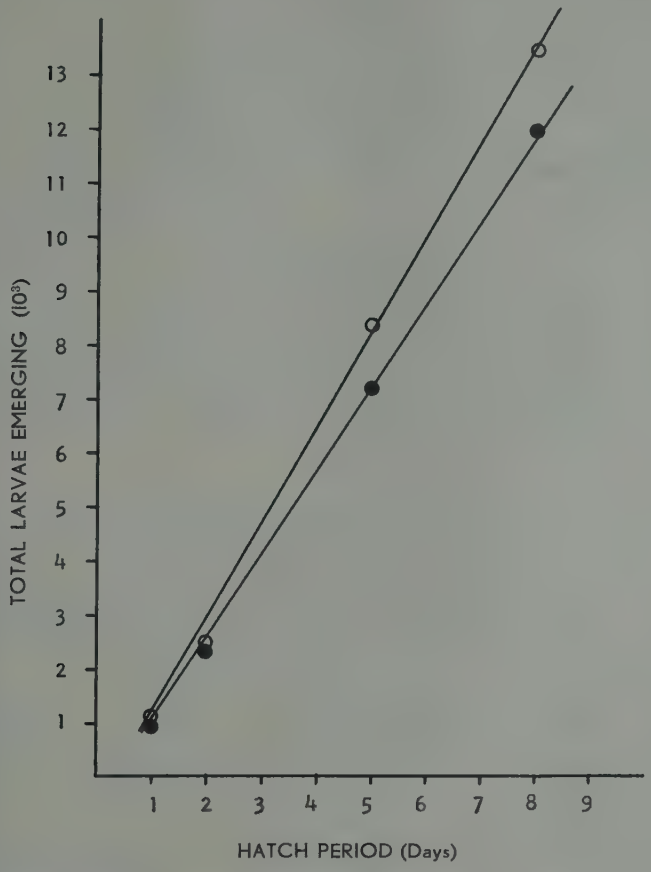

Figure 1. Hatch of larvae of *Meloidogyne incognita acrita* from egg masses. Average hatch from 37 egg masses exposed to 10 seedlings —○—; water hatch —●—.

the three species tested, with the exception of one trial with *M. hapla* (Method I) in which the increase was several times greater. In general, the effect could be observed only with healthy and vigorous seedlings, and when bacterial and fungal action was low.

When the hatch for each species is plotted against time (Fig. 1, 2, 3) an essen-

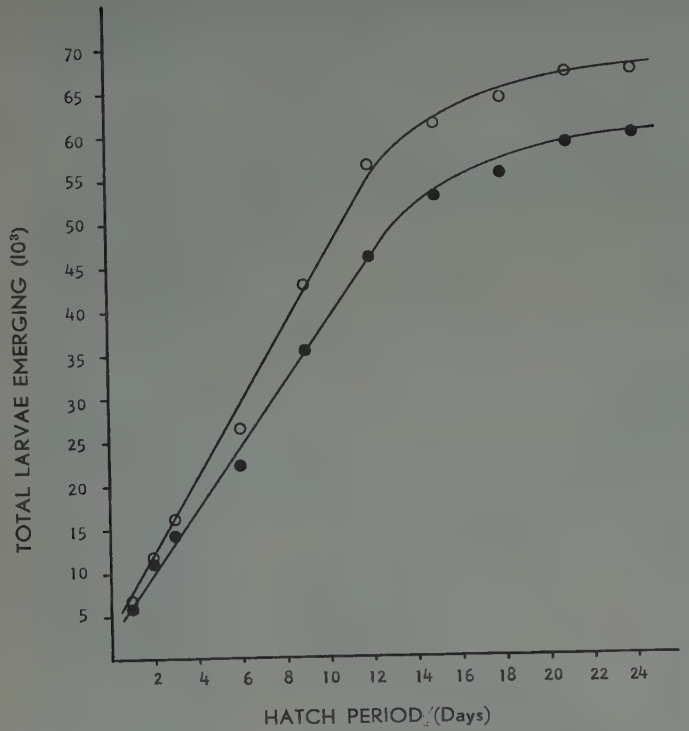

Figure 2. Hatch of larvae of *Meloidogyne hapla* from an aliquoted suspension of egg masses. Average hatch when exposed to 15 seedlings —○—; water hatch —●—. Seedlings were replaced on the 6th, 12th and 18th day.

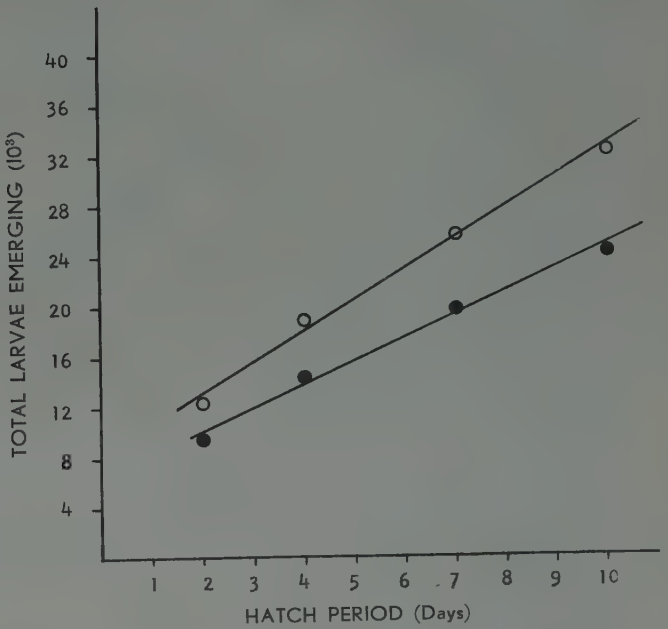

Figure 3. Hatch of larvae of *Meloidogyne javanica* from an aliquoted suspension of egg masses. Average hatch when exposed to 15 seedlings —○—; water hatch —●—.

tially linear relationship is obtained for tests with and without seedlings over a ten day period. Emphasis has been directed toward the steady-state situation rather than toward occurrences in the initial and final stages of hatching or the maximum possible hatch which can be studied in part by the methods used for *Heterodera,* (FENWICK, 1951, FENWICK & WIDDOWSON, 1958, WINSLOW, 1954). With the three species the hatching curves with and without seedlings diverge indicating an increased rate of hatch in the presence of root exudates. Not only is the number hatching per unit time increased, but also a greater final hatch is obtained with seedlings, as shown in Fig. 2, where hatching was continued for a much longer period. Though the hatching curves with and without seedlings become non-linear after 12 days, the hatch rates approach zero concurrently; it is unlikely that greater prolongation of the test would result in equal final hatches. Consequently the effect of the seedlings is to provide a factor or factors whereby larvae are able to hatch that could not do so otherwise.

ZUSAMMENFASSUNG

Die Schlupfreaktion von Meloidogyne-*Arten zu Wurzelausscheidungen von keimenden Tomatensamen*

Mit keimenden Tomatensamen der Sorte "Rutgers" ergab sich gegenüber Kontrollversuchen in Wasser eine deutliche Steigerung der aus Eimassen von *Meloidogyne hapla, M. incognita acrita* und *M. javanica* schlüpfenden Larven. Ferner konnte gezeigt werden, dass durch Wurzelausscheidungen von Tomatensämlingen mehr Larven zum Schlüpfen gebracht werden als ohne sie.

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THE MORPHOLOGY OF THE BRASSICA ROOT EELWORM *HETERODERA CRUCIFERAE* FRANKLIN, 1945

BY

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OOSTENBRINK & DEN OUDEN (1954), COOPER (1955) and MULVEY (1957) have drawn attention to various structures present in the vulval cone of *Heterodera* spp. which can be used taxonomically. The morphology of other stages has been studied, in *Heterodera rostochiensis* by O'BRIEN & PRENTICE (1930), *H. schachtii* by RASKI (1949) and *H. trifolii* by MULVEY (1959). None of these however made use of serial sections, but relied on observing the internal anatomy from whole mounts or hand sections.

The most detailed work on the histological anatomy of any member of the subfamily Heteroderinae Filipjev, 1934 is that of ELSEA (1951) on *Meloidogyne hapla.*

The production of a gelatinous matrix by the adult female in most species is an interesting feature of the subfamily. Its function is not clear and little was known about its origin and development until MAGGENTI & ALLEN (1960) showed that it is produced in *Meloidogyne* by a set of rectal glands. BIRD (1958) studied the chemical nature of the egg sacs of two species of *Meloidogyne* and postulated, as did NAGAKURA (1930), that it was of uterine origin.

H. cruciferae was first described by FRANKLIN (1945) who distinguished it from *H. schachtii* by the difference in the length of the larvae, the small cysts with a less prominent vulval cone and by host range. JONES (1950) gave an account of the morphology of the cyst and also compared the larval measurements of *H. cruciferae* with those of *H. carotae* Jones, 1950.

FRANKLIN (1951), reviewing the genus *Heterodera,* illustrated the male tail and spicules. MILES (1951) doubted the validity of the specific status of *H. cruciferae* and considered separation from other species, especially *H. schachtii,* impossible on strictly morphological grounds. OOSTENBRINK & DEN OUDEN (1953) and COOPER (1955) however have shown that positive identification can be made by the use of characters present in the cyst.

The present work examines the structures found in females of *H. cruciferae* before cyst formation and studies the production of the gelatinous matrix by means of serial sections.

MATERIALS AND METHODS

A supply of cysts of *H. cruciferae* was obtained from Rothamsted Experimental Station, England. Seedlings of cabbage and Brussels sprouts, which had been grown in sterile soil, were transplanted into pots containing sterile soil each inoculated with approximately 100 cysts.

Specimens were either taken from the roots or small portions of root with a nematode attached were excised and were treated in one of three different ways. One group was killed in water by gentle heat, transferred to F.A.A., taken through glycerine/alcohol mixtures and mounted in glycerine for examination *in toto*. A second group was killed in the same way, but was transferred to 10 % neutral formalin. These were washed in running water and then passed through cellosolve and isobutyl alcohol to paraffin wax (M.P. 56° C) using the method of ELSEA (1951). Transverse and longitudinal serial sections were cut at 6 μ and stained with Heidenhain's Haematoxylin (formulation of BAKER & JORDAN (1953)), differentiated in picric acid and mounted in Permount.

The third group comprised a few individuals, each of which had already produced a gelatinous matrix. These were placed in water and the matrices removed by means of a bamboo splinter. The nematodes were killed by gentle heat after four days, during which period further gelatinous material had been produced. Processing and sectioning was then done as for the second group. Sections of this batch were subjected to the Periodic acid-Schiff's (P.A.S.) reaction, since it seemed probable that the gelatinous matrix would contain a P.A.S.-positive substance. To ensure that no preformed aldehydes were present in the sections, non-oxidised controls were used, distilled water being substituted for the periodic acid. The whole test was carried out in accordance with the procedure used by BONHAG (1955).

An attempt to inoculate half radishes and slices of turnip using the technique devised by WILLIAMS (1957) proved unsuccessful due to the breakdown of the plant cells long before the development time of the nematodes had been completed.

RESULTS

The Cuticle

The cuticle is divided into two regions (Fig. 3A), comparable with the exo- and endo-cuticle seen by WIESER (1953) in sections of *H. gottingiana, H. schachtii, H. avenae* and *H. rostochiensis*. The exocuticle is not a smooth stratum, but is heavily ridged, giving the surface of the body wall a zig-zag pattern (Fig. 3B). It can be distinguished because it is stained by haematoxylin, but is even more clearly defined in sections subjected to the P.A.S.-reaction. It contains a P.A.S.-reactive substance which may be similar to the gluco-protein (Mucoid) found in the cuticle of *Ascaris lumbricoides* by CHITWOOD (1936).

The endocuticle appears as a smooth band underlying the exocuticle and is free from P.A.S.-reactive substances, except for a very thin basal lamella, where it lies on the hypodermis. In sections stained with haematoxylin, this layer appears

to be much thicker, so that in fact there may be two layers, the inner one of which contains a polysaccharide.

In the young adult females there is a thin transparent skin overlying the exocuticle and although it is clearly associated with it, there is no conformation on its inner surface corresponding to the ridges on the body wall. It frequently separated during sectioning and when some individuals were being removed from the roots, it broke free, being apparently unattached. It is not stained by haematoxylin and does not contain any P.A.S.-reactive substance. No evidence can be put forward to show whether it is a cast larval skin or a secretion of the cuticle, but it seems possible that from this structure develops the "subcrystalline layer", which is a feature of the cyst and is illustrated by FRANKLIN (1945).

The Hypodermis

The hypodermis underlies the cuticle and occurs as a syncytial layer containing large, round nuclei, which stain readily (Fig 2D). It is similar to that found in *Meloidogyne* by ELSEA (1951), being narrow in the cephalic region, relatively broad in the equatorial region and occupying the whole area around the vagina in the posterior region of the body.

Only oblique sections of the head were obtained, due to the deviation of this portion from the main axis of the body, rendering them almost impossible to interpret. The arcade cells round the stoma, described by Elsea, appear however to be present.

The Somatic Musculature

The somatic musculature is vestigial in the adult female. This is not surprising, since the saccate nature of the body situated inside a root must preclude any great movement, though LINFORD (1937) has shown that the head of mature females of *Meloidogyne* is mobile.

The Digestive System

There is a slender stylet about 22 μ in length. The corpus, median bulb and overlapping oesophageal glands remain as distinct structures (Fig. 1) in the adult female, but the intestine is not so discrete, filling the entire central volume of the body.

The corpus (Fig. 2A) is lined with cuticle surrounding a circular lumen along its anterior length. Posteriorly the cuticle thins out and a triradiate lumen occurs, which continues throughout the median bulb. The median bulb (Fig. 2B) is highly muscular and contains nuclei of the three types, marginal, radial and nerve, described by CHITWOOD & CHITWOOD (1950).

The oesophageal glands occupy a prominent position in cross section, but cannot be distinguished as three separate entities. A striking feature is the presence of only one large nucleus, situated in the posterior part of the gland (Fig. 2C), instead of the usual three.

The intestine is a syncytium containing nutrients. It has an irregularly shaped lumen along its fore-part, but this cannot be traced in the posterior half of the body and the rectum is poorly defined. No evidence of branching of the lumen, as reported by Elsea in *Meloidogyne,* is apparent. The intestine contains much food material, so it is suggested that in the adult female, this structure becomes a storage organ rather than a digestive centre. Because a large number of eggs is produced in a relatively short time, a great deal of readily available nutrients must be required.

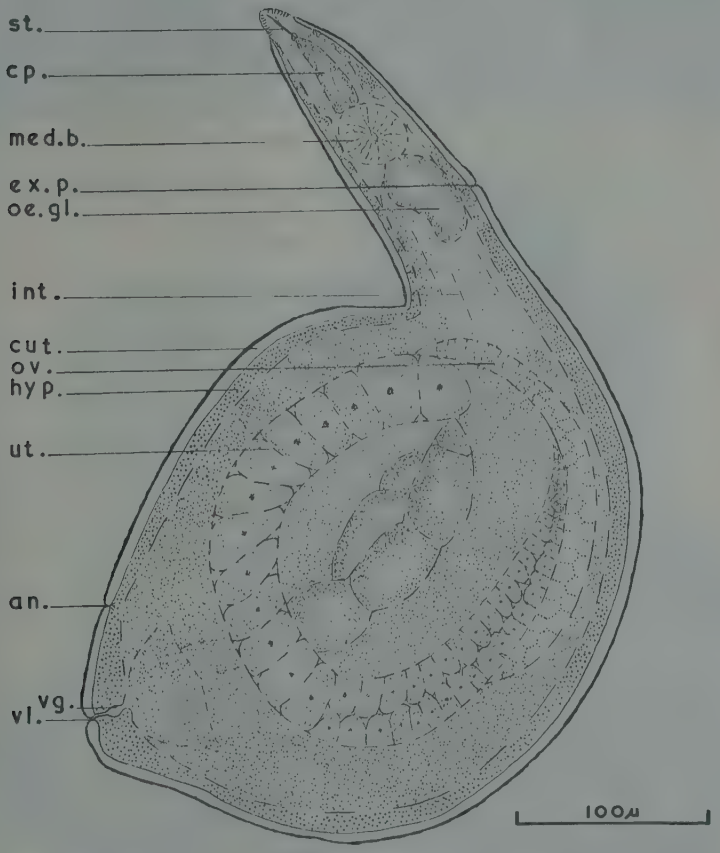


Fig. 1. Young adult female. an. = anus; cp. = corpus; cut. = cuticle; ex. p. = excretory pore; hyp. = hypodermis; int. = intestine; med. b. = median bulb; oe. gl. = oesophageal glands; ov. = ovary; st. = stylet; ut. = uterus; vg. = vagina; vl. = vulva.

The Excretory System

Few details of the excretory system could be seen in the sections. The excretory pore lies in a position approximately level with the middle of the oesophageal

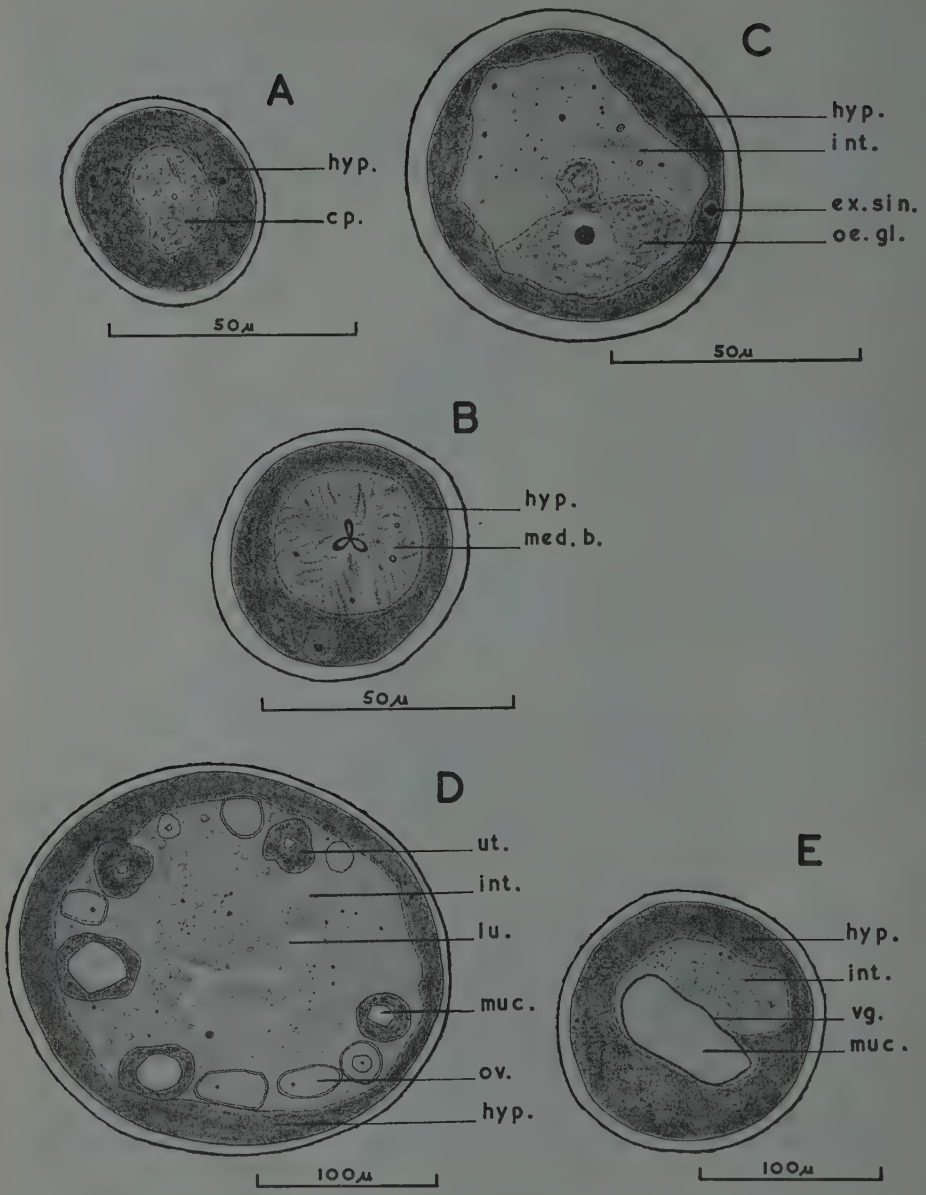
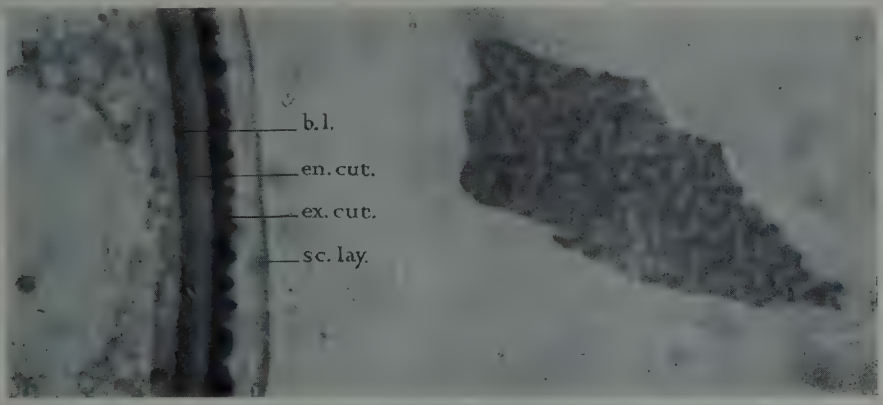


Fig. 2. Transverse sections through various regions of a young adult female. A. Through the corpus. B. Through the median bulb. C. Through the oesophageal glands. D. Through the equatorial region. E. Through the vagina.

ex. sin. = excretory sinus; lu. = lumen; muc. = mucus. Other legends see Fig. 1.

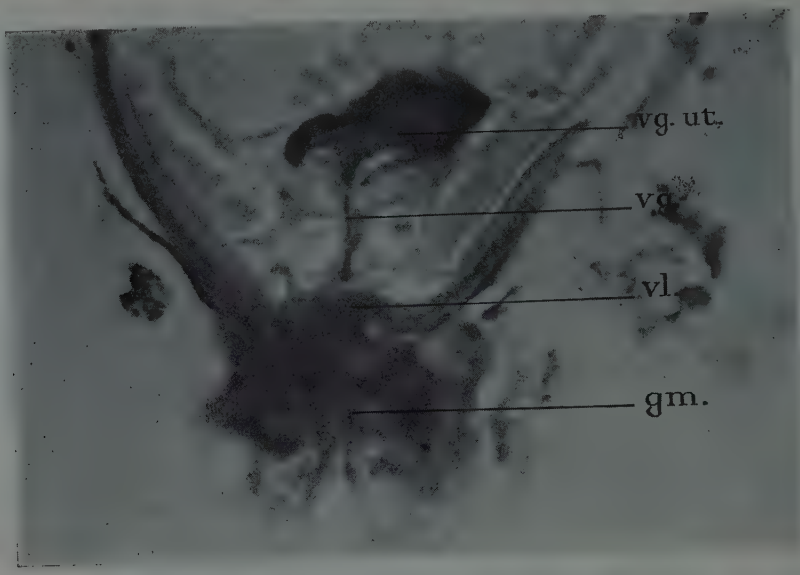


A B

Fig. 3. A. Longitudinal section through the cuticle (1600 x). b.l. = basal lamella; en. cut. = endocuticle; ex. cut. = exocuticle; sc. lay. = "subcrystalline layer".
B. Surface pattern of the cuticle (1600 x).

Fig. 4. Longitudinal section through a mature female of *H. schachtii* (120 x). muc. = mucus; gm. = gelatinous matrix.

A



B

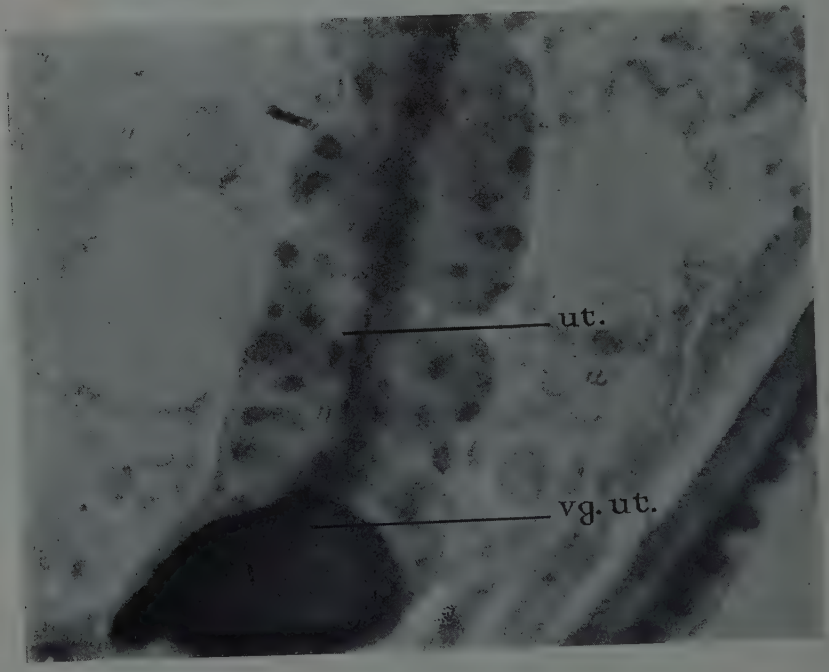


Fig. 5. A. Longitudinal section through the posterior region of the young adult female (640 x). gm. = gelatinous matrix; vg. ut. = vagina uterina.
B. Longitudinal section of a young adult female showing part of the uterus (1600 x).

glands (Fig. 1). A duct leads from the pore into the hypodermis, but its course soon becomes obscure. At the same level as the large nucleus in the oesophageal glands, there is a smaller nucleus (Fig. 2C) but still relatively large. This is the nucleus of the excretory sinus. Similar nuclei, which may serve an excretory function, occur in the hypodermis in a lateral position at the level of the corpus and throughout the hypodermis, posterior to the sinus.

The Reproductive System

The female reproductive organs occupy a position between the hypodermis and the intestine (Fig. 2D). The spaces between the coils of the uteri and ovaries are filled by intestinal tissue and while the gonads are embedded in this tissue, they are not completely surrounded by it.

(a) Ovary. The germinal zone of the two ovaries is seen in cross section to be composed of five oogonia, arranged radially around the germinal stem. No epithelium can be distinguished round this area. In the growth zone, the ovary is bounded by a distinct epithelium and the lumen is filled by a single row of developing ova.

(b) Uterus. The distal part of each of the two uteri is composed of an epithelium of three large cells enclosing a roughly triangular lumen, but in the proximal region the uterine wall is made up of four cells surrounding an approximately square lumen. The nature of the lumen is discussed in the section dealing with the gelatinous matrix.

(c) Vagina. The vagina vera is made up of eight cells arranged in two groups of four, lying one on each side of the lumen, which is lenticular. This organ is heavily reinforced by a single cuticular layer, except in the region where it merges with the uterus, the vagina uterina. The lining is continuous with the external cuticle. The vagina terminates at the transverse vulva.

The Gelatinous Matrix

Preliminary tests indicated that the gelatinous matrix is reactive to the P.A.S.-test, giving a deep magenta colour. Further tests attempted to trace the matrix to its source. By the use of the P.A.S.-reaction it is possible to delineate the path taken by the forming matrix, back through the vagina (Fig. 5A) and along the length of the uteri (Fig. 5B).

Specimens, which were induced to form a second matrix and sections of certain other young adults, show that the lumen of the uterus is large (Fig. 2D) and when stained with haematoxylin appears to be empty. It is, in fact, filled with material, which gives the same reaction to the P.A.S.-reagent as does the egg sac, and is continuous with it. The matrix is not completely extruded as soon as the mucus is produced by the uteri, but is accumulated at the junction of the uteri and the vagina uterina, which becomes greatly dilated to accommodate it, before it is passed through the vagina to the exterior at the vulva (Fig. 2E).

No trace of special glands associated with the rectum can be seen in *H. cruci-*

ferae and all the evidence points to the secretion of the gelatinous matrix by the cells of the uterine wall. Additional evidence has been obtained from sections of *H. schachtii,* which have reached a later stage of development. In these (Fig. 4) the eggs are surrounded by a substance, which is indistinguishable from the gelatinous matrix. It is logical to assume that this material is formed merely by a continuation of the process that is responsible for the production of the external matrix and is retained within the developing cyst.

Pathogenicity

FRANKLIN (1951) thought crop failures among brassicas were probably caused by fungal parasites in addition to nematodes and *H. cruciferae* is not usually regarded as a major crop pest. Sections of roots containing nematodes however, show that giant cell formation, as first described by NEMEC (1910) in sugar beet, takes place and there is considerable rupturing of the cortex as the body of the female dilates. The rapid production of other roots appears to minimise this effect.

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This account is based on a thesis submitted to the University of California for the degree of Master of Science.

ZUSAMMENFASSUNG

Die Morphologie des Kohlzystenälchens (Heterodera cruciferae *Franklin, 1945*)

Die Morphologie des voll entwickelten Weibchens von *Heterodera cruciferae,* namentlich die Beschaffenheit der Epi- und Hypodermis, der Verdauungs-, Ausscheidungs- und Fortpflanzungssysteme, wird beschrieben. Das Nervensystem wird nicht behandelt.

Besondere Aufmerksamkeit wird auf die Bildung des gelatinösen Grundstoffs gerichtet. Es werden Beweise angeführt, die zeigen sollen, dass er aus den Gebärmutterzellen entsteht.

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EXPERIMENTS WITH *XIPHINEMA INDEX* AS A VECTOR OF FANLEAF OF GRAPEVINES

BY

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Fanleaf disease of grapevines was found in California 10 years ago (Hewitt, 1950) and the soil-borne nature of the virus causing it shown 6 years later (Hewitt, 1956). The dagger nematode, *Xiphinema index* Thorne & Allen, 1950, was demonstrated to be a vector of the fanleaf virus in 1958 (Hewitt, Raski & Goheen, 1958). Jha & Posnette (1959) and Harrison & Cadman (1959) have shown that a species of *Xiphinema* occurring in soils of Britain is a vector of the *Arabis* mosaic virus. Breece & Hart (1959) point out the association of *Xiphinema americanum* Cobb, 1913 with the transmission of peach yellow bud mosaic virus to peach.

These reports show the importance of nematodes in the spread of soil-borne viruses. There is need for information concerning the relation of virus to the nematode as a vector. This paper reports the results of some studies on the retention of the virus by nematodes, feeding time required for transmission, possible transovarial passage and transmission by larval stages of the nematode. Some of the data in this paper were given at the IX International Botanical Congress (Raski & Hewitt, 1959).

PERSISTENCE OF THE VIRUS IN THE NEMATODE

A test was set up to determine the time dagger nematodes survived in the absence of a host.

Sixty 4-inch pots were half filled with a mixture of one-half sand, one-fourth sandy loam and one-fourth peat moss, fumigated by methyl bromide. The hole in the bottom of each pot was plugged with cotton to prevent nematodes from migrating out or being washed out during watering and yet to permit adequate drainage. A population of *X. index* was obtained by wet screening soil from about roots of healthy St. George grapevine rootings, cleaned by self filtration and divided into lots containing 500 nematodes in all stages. These were added, in water, to the soil of each pot which was then filled with more soil. Each 4-inch pot was placed inside a 6-inch one and the space between them filled with fumigated soil to minimize drying. The soil in both pots was watered similarly.

Thirty of the double pots were watered regularly and the other thirty allowed to dry. At intervals up to 164 days five replicates from the moist series were

wet screened and the number of live *X. index* counted. The dry series was discontinued at 35 days because by then almost all the nematodes had perished. The results of these counts are shown in Table I. The nematodes recovered included larvae as well as adult females.

TABLE I

Survival of Xiphinema index *in sterile soil in the absence of suitable host plants*

No. of days without host plant	Replicate	Nematode Counts 1	2	3	4	5	Average
Moist Series							
12		165	107	147	76	65	112
38		70	131	16	141	33	78
53		53	9	71	15	0	30
71		26	41	13	16	27	25
163		1	16	13	12	14	11
164		14	11	11	6	7	10
Dry Series							
7		275	215	200	144	142	195
35		0	4	0	0	0	1

The transmission of fanleaf virus by nematodes that survived for different periods of time in moist soil was tested in a similar experiment. On 6 June 1958, a suspension of 300 viruliferous nematodes from roots of fanleaf diseased St. George vine was put into the soil of each of fifty 4-inch pots held inside 6-inch ones as in the previous test. At intervals for 30 days the soil from each of ten pots was removed and spread over the surface of another 6-inch pot half-filled with sterilized soil. The soil ball of a rooted, healthy St. George vine was placed directly on the test soil and the pot filled with sterile soil. In the check series (0 days) the nematodes in water were poured directly into 6-inch pots of sterile soil into which healthy St. George rootings had just been transplanted.

On 17 March 1959, all of the treatments from 0 time to 30 days had vines showing fanleaf disease (Table II). The soil from the roots in each pot was wet screened and heavy populations of *X. index* were found in every replicate.

TABLE II

Persistence of fanleaf virus in Xiphinema index *in the absence of host plants*

No. of days in sterile soil without host	No. of healthy St. George rootings Inoculated with nematodes	Infected with fanleaf
0	10	6
1	10	5
3	10	5
7	10	7
14	10	7
30	10	4

TRANSOVARIAL PASSAGE OF THE VIRUS TO PROGENY

Viruliferous nematodes were wet screened from the soil about fanleaf infected plants and gravid females hand-picked. These females were kept in a thin layer of distilled water or in water with a small quantity of sand, until eggs were deposited. In some cases the eggs were not disturbed until the larvae hatched. These larvae were then transferred to healthy plants. Ten such larvae were transferred individually to separate test plants. Five larvae were transferred to each of four other test plants. Single eggs were transferred directly to each of ten plants. Healthy, single-bud St. George cuttings rooted in sand were used as test plants.

These transfers were made between 25 June and 5 July 1958. On 27 June, each of ten healthy St. George rootings were inoculated with 500 nematodes from the population used above to test for the presence of virus. By 9 October 1958, three plants in this series showed positive symptoms of fanleaf. By 17 March 1959, all ten plants were infected. Six uninoculated plants remained virus-free throughout the test period.

None of the plants in the series inoculated with eggs or larvae was found to be infected with fanleaf virus. After the completion of the test, nematode populations were checked by wet screening the soil of each separate pot. Many nematodes were present in nine of the ten pots inoculated with single larvae, four of the ten inoculated with single eggs, one of the four with five larvae each, and all the ten with 500 nematodes initially. No nematodes were found in the soil of the six check plants.

VIRUS TRANSMISSION BY VARIOUS STAGES OF X. INDEX

Nematodes, wet screened from the soil about the roots of a fanleaf infected plant were hand-picked into lots by stages and transferred individually or in groups of five of ten of the same stage of development to healthy St. George rootings. Males are extremely rare in greenhouse populations and the nematodes tested here were adult females or larval forms presumably destined to be females. Recently hatched, judged here to be second stage, larvae have a characteristic small size and a conical tail. Pre-adult fourth-stage larvae are also distinctive in the digitate tail shape, large size and developing gonad. The other immature forms, intermediate in size and with a conical tail shape, were listed separately as third-stage larvae.

When single nematodes were tested, the specimen was washed off the handling pick directly onto the soil ball of a test plant. If five or ten were used, the nematodes were transferred to small beakers of tap water, then poured as a suspension into a hole in the root ball of the test plant. The inoculations were made on 9 June 1958, and final assessments of virus symptoms and nematode populations were made in May, 1959. Table III shows the results of these inoculations.

TABLE III

Fanleaf virus transmission by various stages of Xiphinema index

No. of Nematodes Per Plant	Stages of Nematode Development	No. of St. George Rootings Inoculated	Nematodes Failed	Nematodes Reproduced	Virus was Transmitted
1	2nd stage larva	10	9	1	1
5	2nd stage larva	10	1	9	1
10	2nd stage larva	10	1	9	0
1	3rd stage larva	20	7	13	3 [1]
1	Pre-adult larva	10	4	6	0
5	Pre-adult larva	10	1	9	1
10	Pre-adult larva	10	0	10	4
1	Adult female	10	0	10	0
5	Adult female	10	0	10	2
10	Adult female	10	0	10	2
0	——	10	—	—	0

[1] In one instance transmission occurred, but the single nematode failed to reproduce. In all other records of transmission the nematodes established and reproduced.

TRANSMISSION WITHIN LIMITED TIME EXPOSURE TO HEALTHY TEST PLANTS

A suspension containing about 600 viruliferous nematodes of various stages was poured into the root zone of a new, single-bud, St. George cutting rooted in a 4-inch pot to provide test plants with limited root systems. One series of ten pots was left in place continuously. Five other series were removed at intervals from 1-28 days. The roots of these plants were washed clean of soil and subjected to a vigorous stream of water to eliminate the nematodes. As shown in Table IV this was accomplished only in one to three replicates in each series but the results indicate that transmission of the virus may occur within 24 hours of the nematodes being introduced to roots of healthy plants.

TABLE IV

Transmission of fanleaf virus by Xiphinema index *in limited time exposures to healthy plant roots*

No. of days on St. George Rootings Before Removal	No. of Replicates	Nematodes Present at End of Test		Nematodes Absent at End of Test	
		Healthy	Fanleaf	Healthy	Fanleaf
1	10	8	0	2	1
3	10	9	2	1	1
7	10	9	6	1	1
14	10	8	4	2	2
28	10	7	5	3	3
Continuously	10	10	6	0	0
Checks	10	0	0	0	0

DISCUSSION

It is evident that fanleaf persists in its vector, *X. index,* for at least 30 days during which the nematodes can survive free of host-plant roots and still remain capable of transmitting the virus. Persistence suggests that the fanleaf virus is not a contamination of the feeding mechanism but is intimately associated with the digestive system of the nematodes. The implications of such persistence is of importance in formulating control measures particularly in view of the ability of single nematodes to transmit the virus.

Transovarial passage of the virus to succeeding generations is not indicated in the tests. However, this cannot be ruled out conclusively until more detailed tests are made to ensure the particular females depositing the eggs tested do in fact carry the virus.

ZUSAMMENFASSUNG

Versuche mit Xiphinema index *als Vektor des „fanleaf"-Virus der Weinrebe*

Versuche zur Übertragung des bodengebundenen „fanleaf"-Virus der Weinrebe durch das Nadelälchen *Xiphinema index* zeigten, dass das Virus in dem Nematoden wenigstens 30 Tage hindurch persistent ist und durch einzelne Nematoden, Larven und erwachsene Tiere, übertragen werden kann. Die Übertragung kann innerhalb von 24 Stunden beendet sein, nachdem die Nematoden in die Wurzeln gesunder Pflanzen eingedrungen sind. Übertragung über das Ei auf die Nachkommenschaft der Nematoden wurde nicht beobachtet.

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PARASEINURA (NEMATODA: APHELENCHOIDIDAE), A NEW GENUS FROM EAST PAKISTAN

BY

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Large numbers of a species of nematode closely resembling *Seinura* Fuchs, 1931, were collected in October, 1959, from a rotting banana inflorescence lying on the ground. Females were about four times as numerous as males. Frequent examination of banana roots nearby and of soil samples in adjacent localities failed to disclose any further specimens. Because this nematode has a small gubernaculum and differs in the morphology of the stylet and lip region from all species of *Seinura,* a new genus, *Paraseinura,* has been designated for it.

The nematodes were studied in the living condition. Measurements and drawings were made of specimens preserved in 5 % formalin, but the measurements of the type specimens were of glycerine jelly mounts.

PARASEINURA N.G.

Diagnosis: Family Aphelenchoididae. Head continuous with body contour; lip bases opaque and sclerotized; stylet without basal knobs but with small attachment points; tip of stylet jointed; female reproductive system monodelphic, with short post-vulvar uterine sac, ovary outstretched; single outstretched testis; equal thorn-like spicules with prominent rostrum and apex; small gubernaculum; tail conical-filiform, with exceedingly fine posterior portion.

Type species: *Paraseinura musicolus* n. sp.

The genus differs from *Seinura,* to which it is most closely related, in the presence of a gubernaculum, sclerotized lip bases, and a jointed stylet tip.

PARASEINURA MUSICOLUS N.SP.

Fig. 1, A-G

20 ♀♀: L = 640-850 μ (760 ± 22 μ); a = 32-42 (37.3); b = 8-11.4 (9.1); c = 2.8-4.6 (3.3); V = 52.8-63 % (56.3 %). Stylet 22-25 μ. Tail 19.2-27.2 (22.7) anal-body-diameters long or 2.1-2.6 vulva-anus distance.

20 ♂♂: L = 490-660 μ (567 ± 15 μ); a = 38-49; b = 7.2-9.3 (8.1); c = 3-5 (4). Stylet 16-17 μ. Tail 10.5-17 (13.9) anal-body-diameters long.

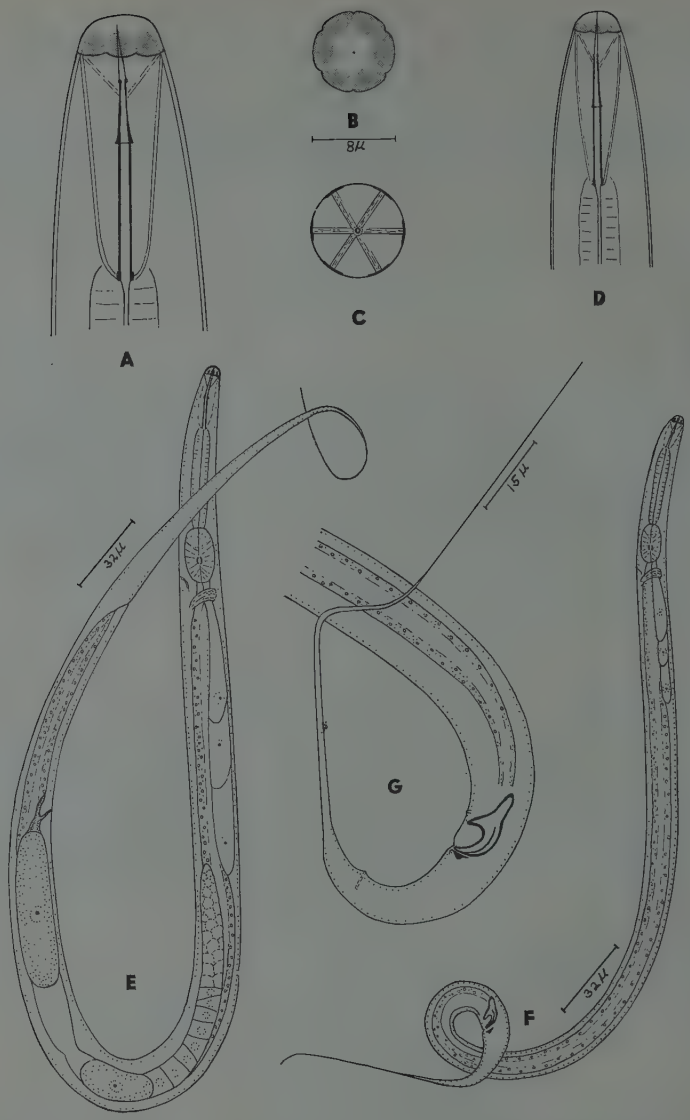

Fig. 1. *Paraseinura musicolus* n.g., n.sp. A. Female head. B. *En face* view of female head. C. Optical section at base of lips. D. Male head. E. Entire female. F. Entire male. G. Male tail.

Holotype female: L = 690 μ; a = 41; b = 8.9 (bulb) or 4 (base of glands); c = 3.6; V = 53.3 %. Stylet = 23 μ. Tail = 20 anal-body-diameters long. Body pale and delicate in appearance, tapering toward anterior and posterior ends. Head

usually inclined slightly dorsally in death. Cuticle exceedingly finely striated; striae less than 0.5 μ apart. Lateral alae indistinct. Head region continuous with body contour, somewhat truncate anteriorly. Lip region prominent due to opaque lightly-sclerotized lip bases, ridged and protruding, greenish in life and refractive after clearing in glycerine but of a different shade of color than that of stylet. Fibre-like attachments from each lip base running at a 45° angle toward stylet and apparently joining a delicate sheath around tip of stylet. Sclerotized stylet guide or cheilorhabdions absent. Stylet 23 μ long or 30 % of the esophageal length to the middle of the median bulb, without basal knobs but with small distinct sclerotized attachment points for stylet protruder muscles. Median part of stylet 6 μ long. Tip of stylet 5 μ long, wider at base than the anterior of the median part and inclined dorsally, though with aperture on ventral side; tiny but distinct sclerotized beads, located slightly asymmetrically, are found at sides of tip base at juncture with median part, setting tip off strikingly from rest of stylet (Fig. 1, A). Protruder muscles of stylet fine and thin. Middle bulb of esophagus elliptical, 22 $\times$ 10 μ, with sclerotized crescentic valve plates at two-thirds of bulb length; anterior third of median bulb granular and somewhat vacuolated; posterior two-thirds of bulb muscular. Esophageal glands distinct, free on dorsal side. Lumen of intestine broad and distinct. Excretory pore opposite base of mid-bulb. Ovary single, anterior, outstretched, extending to esophageal glands; oocytes in two rows in ovary. Tiny collapsed post-vulval uterine sac, about one-half body diameter long. Vagina and uterine sac sclerotized. Posterior part of uterus serves as spermatheca, set off by constriction from oviduct. One ovum with shell present in uterus, 60 $\times$ 15 μ. Tail conical-filiform, without marked division between the two parts, 20 anal diameters long or 2.1 times the vulva-anus distance. Body curved slightly ventrally in death.

Allotype male: L = 540 μ; a = 41.6; b = 7.3 or 4.8; c = 3.7. Stylet = 17 μ. Tail = 14.7 anal-body-diameters long. Anterior of body similar to female but stylet shorter (17 μ) and division into three parts less conspicuous; median part 5 μ long, tip 3.5 μ long. Striation and lateral fields not observed. Esophageal bulb 16 $\times$ 7 μ. Testis single, outstretched, inconspicuous. Spicules paired, shaped like a rose-prickle; tips of limbs joined posteriorly; dorsal limb 17 μ long, with prominent rounded apex one-third its length; ventral limb 8 μ long, highly arcuate, with ventral rostrum; distance between apex and rostrum 8.5 μ; dorsal and ventral limbs connected anteriorly by a sclerotized bar for most of distance between them, continuous with a thin septum near the apex. Internal division of spicule, seen at certain angles, seemingly represents bulging lateral margin of spicule shaft. Small gubernaculum or apophysis posterior to cloacal wall, somewhat triangular in shape but rounded at apex; of same color and refractiveness as spicules. Three pairs subventral papillae: one small pair at level of proximal end of spicules; one tiny indistinct pair adanal or just behind anus; and one prominent pair at posterior of conical part of tail. Tiny lateral papillae, probably representing phasmids, about one anal-body-diameter posterior to anus. Tail distinctly divided into conical and

filiform portions, with extremely long fine tip. Tail in death characteristically coiled once.

DISCUSSION

The stylet of *Paraseinura musicolus,* with its distinctive tip, seems to be unique in the Aphelenchoidea. The fibre-like connectives between the lip bases and the stylet sheath are also unusual. Several species of the Aphelenchoididae have been described as having a small apophysis in the normal position of the gubernaculum, e.g., *Rhadinaphelenchus cocophilus* (Cobb, 1919) J. B. Goodey, 1960. This structure would seem to represent an incipient gubernaculum, but is not of phylogenetic importance. In the form and length of the tail, in the shape of the spicules, and in the pattern of papillae on the male tail *Paraseinura musicolus* is definitely more related to the species of *Seinura,* especially *S. citri* (Andrássy, 1957) J. B. Goodey, 1960, of which only females are known. However, the female of *P. musicolus* differs from *S. citri* in its much larger size and in the tripartite stylet and sclerotized lip region.

Holotype female: Collected on October 25, 1959, by R. W. Timm. FACP Type Collection No. 3.

Allotype male: Same data as holotype. FACP Type Collection No. 4.

Paratypes (male and female): Personal collection, slides P 1-5; vial deposited with Nematology Division, U.S.D.A., Beltsville, Md., U.S.A.

Type habitat: Rotting inflorescence of *Musa paradisiaca* L. (cultivated banana).

Type locality: Notre Dame College, Dacca.

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RÉSUMÉ

Paraseinura (Nematoda: Aphelenchoididae), *un nouveau genre de l'Est du Pakistan*

L'auteur donne la diagnose d'un nouveau genre de nématodes trouvé dans l'inflorescence pourrie d'un bananier. Le nouveau genre se différencie de *Seinura* par la présence d'un gubernaculum, de bases sclerotisées des lèvres, et l'absence d'un bouton basal du stylet. *Rhadinaphelenchus cocophilus,* qui possède également un gubernaculum, se différencie par la structure de la region labiale et par la possession d'un "vulval flap" et d'un "bursal flap".

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PARATYLENCHUS MARYLANDICUS, N. SP. (NEMATODA: CRICONEMATIDAE) ASSOCIATED WITH ROOTS OF PINE [1])

BY

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During the summer of 1959, several soil samples were collected from about the roots of Virginia pine and were found to contain a large species of *Paratylenchus* having a very long stylet. Further examination revealed this species to be new.

This species appears in many respects to be intermediate between the genera *Paratylenchus* Micoletzky, 1922 and *Cacopaurus* Thorne, 1943. However, it is closer to the genus *Paratylenchus* and is described below as a new species of this genus.

Paratylenchus marylandicus n. sp.

(Fig. 1)

Females (15) L = 390 μ (340-420 μ); a = 21.9 (19.7-23.6); b = 3.6 (3.2-4.1); c = 10 (8.8.-12) V = 75 (71-79); stylet = 66.7 μ (62.8-71.4 μ).

Holotype (Female): L = 390 μ; a = 21.8; b = 3.5; c = 10; V = 76; stylet = 66.6 μ.

♀ *Description:* Cuticle marked by fine transverse striae about 1 π apart interrupted by a lateral field having 4 lines and occupying about $^1/_5$ the body diameter. Lip region a truncated cone, not offset from the body contour, unstriated. Four lips, which upon fixation often become expanded. Amphids not observed. Excretory pore conspicuous, located on the ventral surface anterior to the basal bulb of the esophagus in the region of the nerve ring.

Buccal cavity small, lacking a sclerotized framework. Stylet long, flexible slightly curved in heat-relaxed and fixed specimens. Stylet knobs slope anteriorly. Dorsal esophageal gland opening into lumen of esophagus about 5-7 μ behind stylet knobs. Esophagus criconematoid. No cardia observed, the esophagus joined directly to tubular intestine which terminates in an obscure anus. Tail varies in shape but generally tapering with a conical terminus.

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[1]) Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, The State University, New Brunswick, New Jersey.

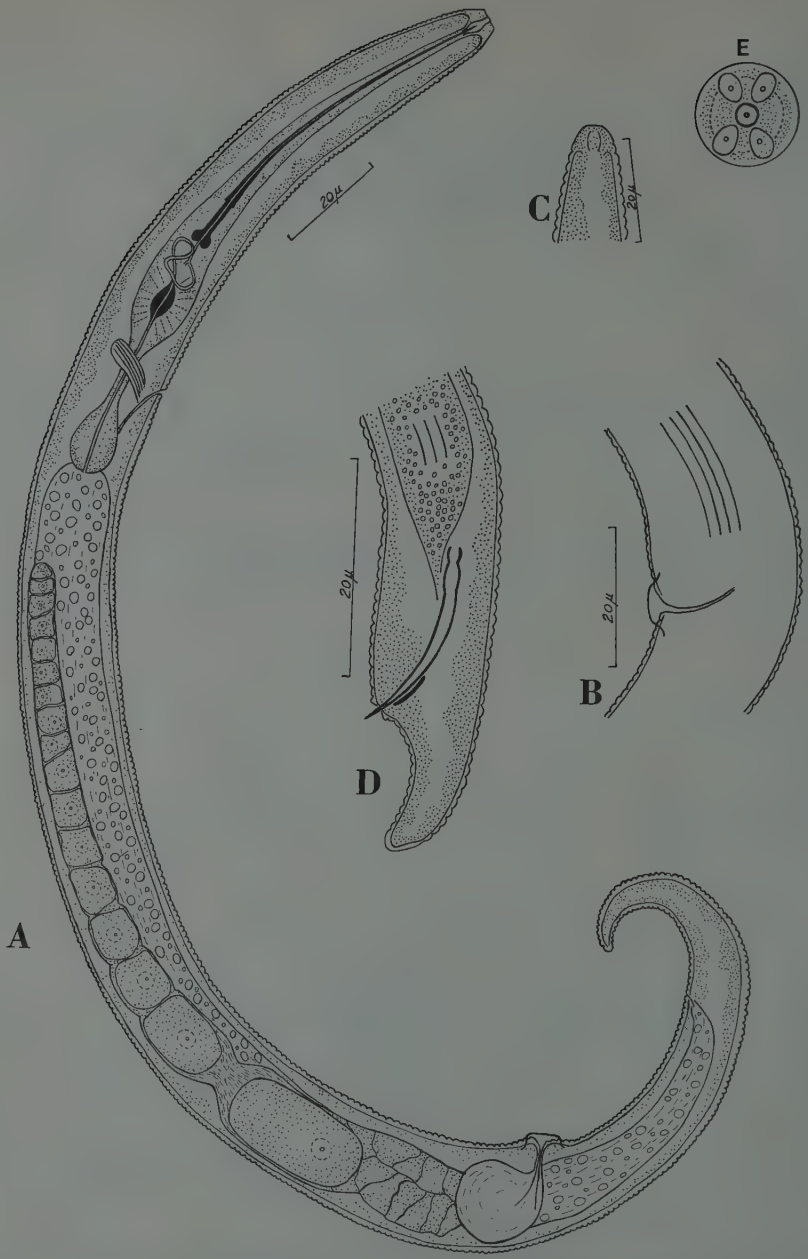

Fig. 1. *Paratylenchus marylandicus* n. sp. A. Mature female, B. Vulvar region showing lateral field. C. Male head. D. Male tail. E. *En face* view of head.

Vulva a conspicuous transverse slit flanked by lateral vulval membranes. Marked reduction in post-vulval diameter accentuates position of vulva. Rounded portion of uterus nearest vulva apparently serves as a spermatheca, occasionally filled with numerous small sperm. No post-larval uterine sac in most individuals. Eggs average 41.4 μ x 13.7 μ.

Allotype (Male): (1) L = 280 μ; a = 23.4; b = ?; c = 15.5; spicule = 19.5 μ.

♂ *Description:* Cuticle marked by striae about 1.5 μ apart interrupted by a lateral field having 3 lines and occupying about $^1/_5$ the body diameter. Lip region a truncated cone marked by a single striation located on the basal portion. Esophagus degenerate, stylet lacking.

Spicula paired, arcuate. Small gubernaculum, slightly curved. No anal sheath surrounding spicula, but the cuticle and hypodermis bulge outward to form an anal body extension. Tail short, appearing stubby, slightly concave and ending in a non-striated conoid terminus. Lower part of single testis filled with small sperm.

Holotype: Female as noted on slide number V 3, Rutgers collection.
Allotype: Male on slide number V 4, Rutgers collection.
Paratypes: 10 females, slides numbered V 2-V 8, Rutgers collection.
Type habitat: Soil about roots of Virginia pine, *Pinus virginiana.*
Type locality: Beltsville, Maryland.

Diagnosis: Females of *P. marylandicus* differ from *P. aciculus* Brown, 1959, which it most closely resembles, as follows: 1) the overall larger size of *P. marylandicus*; 2) presence of lateral vulval membranes in *P. marylandicus*; 3) difference in appearance of vulva and size of post-vulval body diameter; 4) position of excretory pore; and 5) number of lines in lateral field. Males of *P. marylandicus* are distinguished by a short stubby tail, distinctly conoid terminus, and bulge of body about spicula.

The author is indebted to Dr. A. C. Tarjan for confirming the identification of *P. marylandicus.*

ZUSAMMENFASSUNG

Paratylenchus marylandicus *n. sp.* (*Nematoda: Criconematidae) an Wurzeln von* Pinus

Paratylenchus marylandicus wird als neue Art von *Pinus virginiana* beschrieben und abgebildet Das Weibchen unterscheidet sich von *P. aciculus* durch die Körpergrösse, durch das Vorhandensein lateraler Vulvahäutchen, die bei *P. aciculus* fehlen, durch seinen mehr nach hinten gelegenen Exkretionsporus und durch das Vorhandensein von 4 Seitenlinien. Das Männchen von *P. marylandicus* besitzt einen kurzen gedrungenen Schwanz mit einer deutlich konischen Spitze und eine Anschwellung im Körper nahe den Spicula.

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THE OESOPHAGO-INTESTINAL JUNCTION IN THE MONONCHIDAE (ENOPLIDA, NEMATODA)

BY

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While examining many species of the Mononchidae it became evident that the structure of the oesophago-intestinal junction in this family has been misinterpreted. The detailed structure of all the species examined differs from the published figures and descriptions because most workers have worked with fixed material, and also because the oesophago-intestinal junction has not been considered important. In specimens fixed in F.A. 4 : 10 or T.A.F. some of the valve structures described here are often obscure or invisible. For this reason the nematodes must be studied alive, narcotized, or *immediately* after "relaxing" with heat. The valve "funnel" (see later) may become obscure within 15-20 minutes of death. A 0.5 per cent aqueous solution of M.S. 222 Sandoz (a soluble salt of a meta-amino-benzoic acid ethyl ester) is a suitable narcotic for mononchs.

The oesophago-intestinal valves in the Mononchidae are of two types, for which the terms "tuberculate" and "non-tuberculate" are proposed. In this paper *Prionchulus muscorum* (Dujardin) exemplifies the non-tuberculate type, and *Anatonchus tridentatus* (de Man) the tuberculate type. The mononch oesophagus was first described by CHITWOOD & CHITWOOD (1937) and illustrated by further figures in 1950. Its lumen is tri-radiate and its lining sclerotized and greatly thickened locally (see figs. 1 B and 2 C). The thickenings are greatest in the centripetal half of each arm, and are linked with each other by thinner regions. The whole lumen can become circular in cross-section by contraction of the radial muscles to allow the passage of large food particles including whole nematodes. The appearance of this structure in lateral view is shown in figure 2 A, and in dorsal view in figure 2 B. Posterior to the end of the oesophagus there is often a zone of transparent tissue (t.z.), (varying in width in different species) that is usually flanked laterally by three (?) smaller transparent bodies. Behind this is found the sphincter muscle which bears on its posterior face a conical structure (c.o.) of very variable size which projects into the lumen of the intestine. (The sphincter muscle with or without the conical structure is the cardia of some authors. There is no constancy in the use of this term, borrowed from vertebrate anatomy where it refers to the opening of the stomach nearest the heart, and it is meaning-

[1]) At present on study leave from Entomology Division, D.S.I.R., Nelson, New Zealand.

less when applied to a nematode which has neither stomach nor heart). This conical body appears rather "structureless" and is not visible in all specimens. It may be obscured by the dense granules in the intestinal cells, by food in the intestine, or by contraction which brings it up close to the posterior face of the sphincter, or by a combination of these.

THE NON-TUBERCULATE VALVE

(Figs. 1 and 3)

In *Prionchulus muscorum* a cross section of the oesophageal lumen appears as shown in figure 1 B from the base of the stoma to a short distance behind the

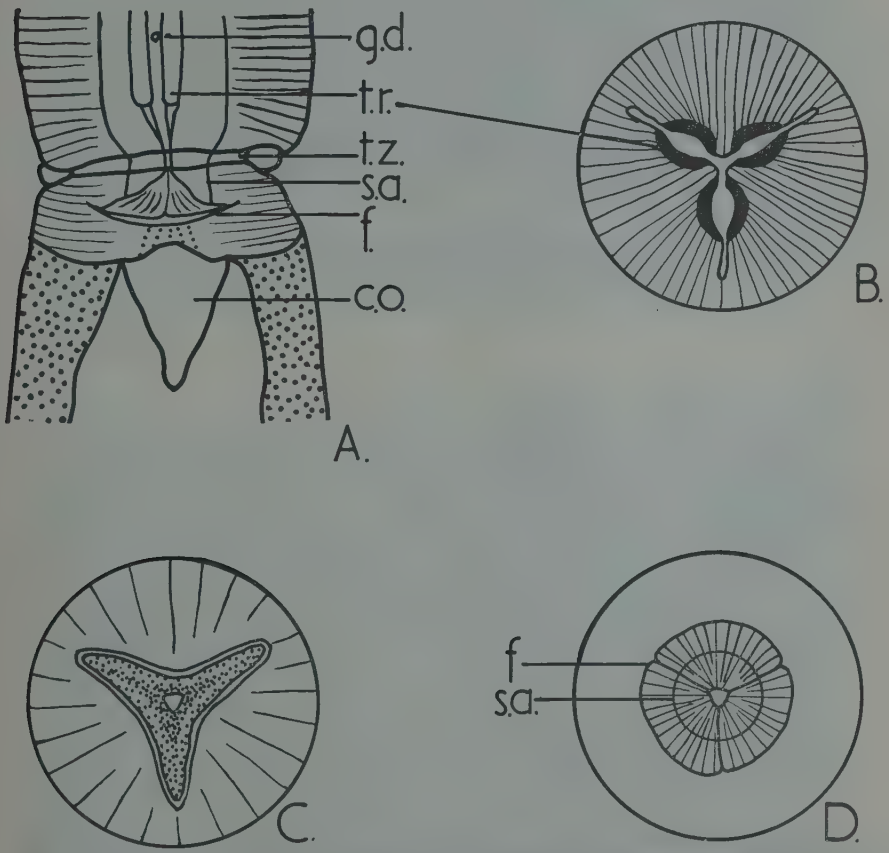

Fig. 1. *Prionchulus muscorum*. A. oesophago-intestinal junction; B. transverse section through oesophagus; C. transverse section through basal part of oesophagus showing the changed form of the sclerotized lining and the narrowing of the lumen; D. transverse section through the sphincter muscle showing the funnel-shaped valve from behind. c.o. conical organ; f. funnel-shaped valve; g.d. sub-ventral oesophageal gland duct; s.a. supporting annulus; t.r. thickened region of oesophageal lining; t.z. transparant zone.

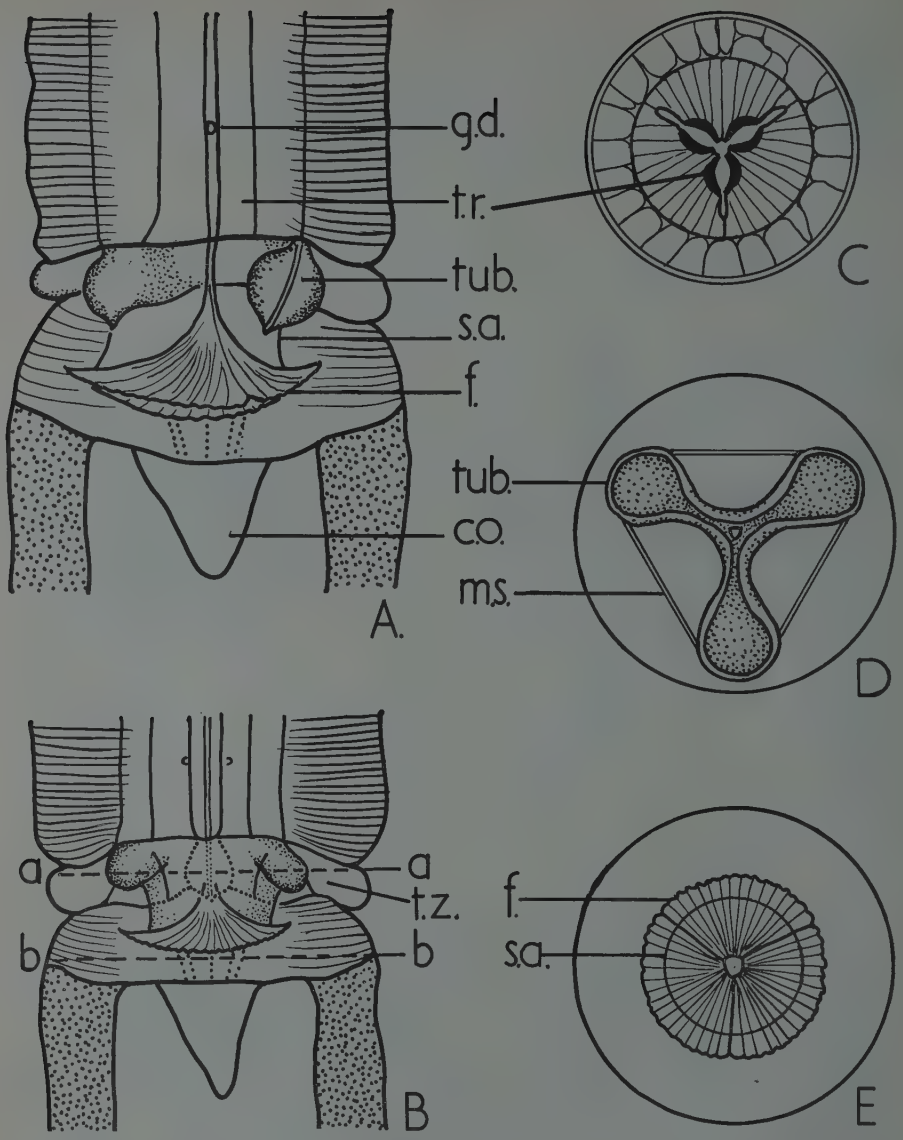

Fig. 2. *Anatonchus tridentatus*. A. oesophago-intestinal junction in lateral view; B. oesophago-intestinal juncion in dorsal view; C. transverse section through the oesophageal region; D. section through oesophagus at level 'a' on fig. B. looking posteriorly showing the hollow tubercles uniting centrally; E. transverse section through the sphincter muscle at the level 'b' on fig. B. looking anteriorly showing the funnel-shaped valve. m.s. muscular (?) strand; tub. tubercles; other lettering as for figure 1.

Fig. 3. Photomicrograph of oesophago-intestinal junction of *P. muscorum* (alive) showing the funnel shaped valve.

Fig. 4. Photomicrograph of oesophago-intestinal region of *A. tridentatus* showing the tubercles and the funnel shaped valve. (Scale applies to both figures).

Photos — C. C. Doncaster.

a b

Fig. 5. Photomicrograph of a moulting larva of *Iotonchus sp.* showing the tubercles (arrow) part way up the oesophagus.

Both photos — C. C. Doncaster.

duct of the posterior subventral oesophageal gland. Here there is an abrupt change in shape as the thickenings disappear and, in cross section, this basal region now appears as in figure 1 C. The arms of the lumen become shorter, which in lateral view appears to narrow where it passes through the end of the oesophagus and the narrow transparent zone (t.z.) into the sphincter muscle. The lumen narrows here and the greater part of its width is made up of a "supporting annulus" (s.a.) which appears to be attached to the much plicated funnel-like structure (f) that forms the valve proper. The lumen expands greatly as it passes into the sphincter muscle, so becoming funnel-shaped. The exact shape varies somewhat with the state of contraction of the sphincter muscle fibres. There are indications that the "funnel" shares the tripartite nature of the stoma and oesophagus linings (Figure 1 D).

THE TUBERCULATE VALVE

(Figs. 2, 4, 5)

In *Anatonchus tridentatus* transverse sections through the oesophagus appear much the same as in *Prionchulus muscorum*. Behind the duct of the posterior sub-ventral oesophageal gland there is no marked change in the apparent width of the lumen as it passes into the transparent zone (t.z.). Within the transparent zone, or in the tissue of the sphincter muscle, or partly in both, the peripheral ends of the three arms of the sclerotized lining become inflated, and greatly thickened, forming fairly conspicuous, hollow tubercles or "knobs" (tub). The actual limits of these tubercles in relation to the transparent zone and the sphincter muscle vary somewhat from specimen to specimen. The cavities of the tubercles are connected in the centre of the oesophagus (see Fig. 2 D). From this point a narrow duct extends posteriorly, and forms the anterior part (or stem) of the tripartite, plicated funnel-shaped valve. The funnel-shaped valve is similar to that found in the non-tuberculate species, and has a similar supporting annulus which appears to be attached to the posterior part of the tubercles. In *A. tridentatus* the posterior rim of the funnel is crenulate, but in *P. muscorum* the rim tends to be smooth.

In moulting specimens of *A. tridentatus* and two undescribed species of *Iotonchus*, the tubercles have been observed part way up the oesophagus in the process of being shed with the rest of the sclerotized lining of the stoma and the oesophagus (Fig. 5).

DISTRIBUTION OF THE VALVE TYPES

The tubercles of the tuberculate type of valve were first reported by DITLEVSEN (1911) in *Anatonchus dolichurus* (Ditlevsen) and later by STEINER (1914) in *Miconchus studeri* (Steiner). MICOLETZKY (1922) did not see these structures in *A. dolichurus*, and regarded those recorded by Steiner as artefacts. Examination of many specimens and species of the Mononchidae has disclosed the following distribution of the two types of valve:

(a) non-tuberculate type present in:

Mononchus Bastian, 1865 (*M. truncatus* Bastian, *M. papillatus* Bastian, *M. kastrolli* Altherr, and three new species from New Zealand).
Prionchulus Cobb, 1916 (*P. muscorum* (Dujardin))
Mylonchulus Cobb, 1916 (*M. brachyurus* (Butschli) and two new species from New Zealand)
Cobbonchus Andrássy, 1958 (*C. mauritianus* (Williams) n. comb., and in three new species from New Zealand).

(b) tuberculate type valves are present in:

Anatonchus Cobb, 1916 (*A. tridentatus* (de Man), *A. dolichurus* (Ditlevsen), and in a new species from Rothamsted).
Iotonchus Cobb, 1916 (*I. acutus* Cobb, *I. brachylaimus* Cobb, *I. trichurus* Cobb, and *I. amphigonicus* (Thorne), a new species from the Philippine Islands, a new species from the Cameroons, and in six new species, one of which may be synonymous with *I. parazschokkei* (Allgen) from New Zealand). *Miconchus* Andrássy, 1958 (*M. studeri* (Steiner)), and in a new species from Falmouth, England, though not seen in *M. trionchus* (Thorne). (These structures are often difficult to detect in specimens which have been mounted on slides for many years).

The type of valve found in the genera *Brachonchulus* Andrássy, 1958, *Granonchulus* Andrássy, 1958, *Judonchulus* Andrássy, 1958, and *Sporonchulus* Cobb, 1917 is not known as material of these genera was not available.

DISCUSSION

The tuberculate type of valve is found in genera in which the tooth or teeth lie in the posterior half of the stoma. Although the teeth in adults of *Anatonchus* spp. may be situated in the anterior half of the stoma, they are basally situated in the larvae. Further, the structure of the valve alone will serve to distinguish members of the genera *Mononchus* and *Iotonchus,* which have become inseparable as at present defined. The funnel-shaped valve, which is often a transient feature of fixed material, is not of systematic significance but the tubercles which show up well in fresh and preserved material appear to be useful.

I thank Mr. C. C. Doncaster for taking the photomicrographs, Prof. G. Thorne for reporting on *I. acutus, I. amphigonicus, I. trichurus* and *M. trionchus,* and Mr. J. R. Williams for reporting on *I. brachylaimus* and for the gift of specimens.

ZUSAMMENFASSUNG

Die Verbindung von Oesophagus und Darm bei den Mononchiden (Enoplida, Nematoda)
Einige morphologische Merkmale der Verbindung von Oesophagus zum Darm verschwinden bei den Mononchiden oft bald nach der Fixierung, besonders die trichterförmige Klappe, die von dem Schliessmuskel umgeben ist. Veröffentlichungen über diesen Bereich stimmen mit der Morphologie der lebenden Nematoden nicht überein. Bei den Oesophagus-Darmklappen der Mononchiden unterscheidet man zwei Typen: 1. den tuberkular-Typ, der durch das Vorhandensein von drei deutlichen

hohlen Bläschen am Hinterende der Oesophagus nahe der trichterförmigen Klappe charakterisiert ist und 2. den nicht-tuberkular-Typ, bei dem die Bläschen fehlen und die Oesophagusführung nahe dem hinteren Ende enger wird. Der tubercular-Typ wurde bei *Anatonchus, Iotonchus* und *Miconchus* gefunden, der nicht-tuberkular-Typ trat bei *Mononchus, Prionchulus, Mylonchulus* und *Cobbonchus* auf.

Mononchus mauritianus Williams 1958 wurde in die Gattung *Cobbonchus* Andrássy überführt.

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REDESCRIPTION OF *MONONCHUS TRUNCATUS* BASTIAN, *M. PAPILLATUS* BASTIAN AND *PRIONCHULUS MUSCORUM* (DUJARDIN) (ENOPLIDA, NEMATODA)

BY

W. C. CLARK

Rothamsted Experimental Station, Harpenden, Herts., England [1])

Three species of the Mononchidae are redescribed; *Mononchus truncatus* Bastian because it is the type species of *Mononchus,* and *M. papillatus* Bastian and *Prionchulus muscorum* (Dujardin) because of the confusion between them (ALTHERR, 1938). Both genera are redefined.

The nematodes examined were collected in England; *M. truncatus,* Falmouth (type locality) and Chippenham Fen; *M. papillatus,* Broadmoor Institution, Berks (type locality), Falmouth, and Woburn, Beds.; *P. muscorum,* Broadmoor Institution, Berks, Falmouth, Woburn, Beds., and Rothamsted. Moss from the Jardin des Plantes, Paris (type locality and habitat of *P. muscorum*) yielded no specimens. Measurements are of eelworms killed by heat, fixed in F.A. 4 : 10 and transferred to glycerine by Baker's method (GOODEY, 1957). The nematodes were examined alive or narcotized because details of the papillae and of the oesophago-intestinal junction become obscured after fixation (CLARK, 1960).

MONONCHIDAE Chitwood and Chitwood 1937 Emend.

Tripyloidea. Cuticle smooth or nearly so; lateral fields absent. Head broad, sub-rounded or truncated; not, or only slightly offset; with six lips and two rings of papillae, the inner ring with six or eighteen, the outer ring with six, ten, or fourteen papillae. Amphids level with the anterior half of the stoma, moderate to small size, pouch-like, with crescentic transverse apertures. Stomal large and wide, heavily sclerotized, tripartite and always armed with single immovable, dorsal tooth; subventral teeth present or absent, smaller or equal to the dorsal tooth. Teeth directed anteriorly or posteriorly. Subventral denticles absent or present in transverse or longitudinal rows, or scattered. Oesophagus very muscular, anteriorly enveloping the posterior end of the stoma, widening slightly posteriorly, always non-bulbar. Nerve ring encircles the oesophagus at one quarter to one third of its length. Dorsal oesophageal gland opens into oesophageal lumen behind nerve ring; one pair of subventral glands open near the posterior end of oesophagus, and the other pair between the other two ducts. Oesophageal lumen tri-

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quetrous, lining sclerotized. Oesophago-intestinal junction with sphincter muscle and lightly sclerotized valve apparatus (usually not visible in fixed specimens). Intestine a simple tube, cells usually with granular contents. Pre-rectum in males only; rectum short, usually straight. Tail long or short, variously shaped, sexes similar or dissimilar; usually with three caudal glands which may or may not discharge through a terminal or subterminal orifice (= "spinneret" of Cobb). Tail usually with paired lateral cuticular pores. *Females* Genital organs mostly paired, opposed, but may be single, either pre- or post-vulvar; ovaries short, reflexed. Only a few eggs in the uteri at a time. *Males* Testes paired, opposed, outstretched. Spicules simple, paired and free. Gubernaculum present; often associated with complex lateral accessory pieces. Supplements in a single ventro-median series, sometimes echinulate, roughly equidistant.

In some species males are rare or unknown.

Found in soil, moss, liverworts, forest litter, and freshwater. Some show a distinct preference for wet soils.

Type genus: *Mononchus* Bastian, 1865.

Mononchus Bastian, 1865

Mononchidae. Stoma large, almost parallel sided, usually with pointed base; armed with a single, stout, anteriorly directed, dorsal tooth, its apex lying in the anterior half. A low, untoothed, ventral ridge may be present. Oesophago-intestinal junction of the non-tuberculate type (CLARK, 1960); i.e. with a stout sphincter muscle surrounding a lightly sclerotized, funnel-shaped extension of the oesophageal lining; never with sclerotized knobs or tubercles. Ovaries paired, rarely single (*M. monohystera* Cobb). Spicules simple, separate, arcuate. Gubernaculum simple, or enveloping the spicules laterally, or associated with complex accessory pieces. Tails mostly short, conoid; or long, conoid at first with a cylindrical end part. Found in freshwater, soil, leaf mould, mosses, and liverworts.

Type species: *Mononchus truncatus* Bastian, 1865.

Mononchus truncatus Bastian, 1865

Figures 1 A-I

Mononchus truncatus Bastian, 1865; p. 101, pl. IX, figs. 25, 26.
Mononchus macrostoma Bastian, 1865; p. 101-102, pl. IX, figs. 29, 30.
Mononchus longicaudatus Cobb, 1893; p. 12 fig. 2.
Mononchus microstoma Cobb, 1893; p. 13.
Mononchus (Mononchus) truncatus Cobb, 1916; p. 195.
Mononchus (Mononchus) macrostoma Cobb, 1916; p. 195.
Mononchus (Mononchus) longicaudatus Cobb, 1916; p. 195.
Mononchus macrostoma var. *filicaudatus* Schneider, 1937; p. 48, figs. 5a, b.
nec. Mononchus macrostoma var. *armatus* Daday, 1897; p. 108, fig. 14. (= *M. dadayi* Micoletzky, 1914 *sp. inquirenda*).
nec. Mononchus longicaudatus Schuurmans Stekhoven & Teunissen 1938; p. 85, figs. 44a, b, not of de Man nor of Cobb.
nec. Mononchus macrostoma Schuurmans Stekhoven & Teunissen 1938; p. 86, figs. 45a, b.

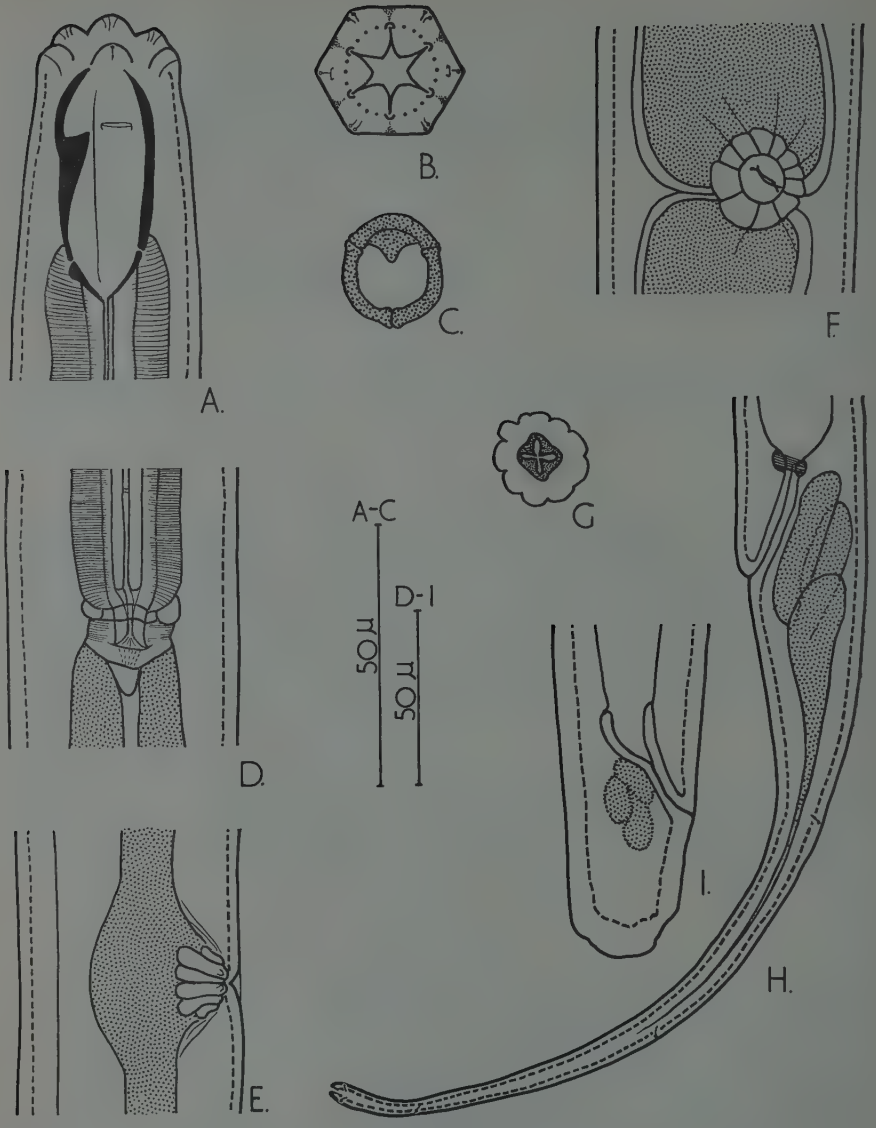

Figure 1. *Mononchus truncatus*. A. Head region; B. *en face* view of head; C. cross section of stoma at the level of the dorsal tooth; D. oesophago-intestinal junction; E. lateral view of the vulva; F. ventral view of vulva; G. sectional view of vagina; H. female tail; I. abnormal female tail.

Falmouth

Neotype ♀ L = 1.92 mm; a = 28; b = 3.9; c = 8; V = ^{14} 55.5 ^{13}
♀♀ (n = 9) L = 1.86 mm; (s = ± 0.143 mm) (range 1.65-2.14 mm)
a = 26-40; b = 3.4-4.3; c = 6.4-8.6;
V = $^{10-22}$ 48.4-55.5 $^{10-15}$

Chippenham Fen

♀♀ (n = 2) L = 1.67-1.85 mm; a = 33-36; b = 3.6-3.8; c = 5.8-6.4;
V = $^{9-11}$ 53.4-54.3 $^{7-12}$

Neotype ♀ from moss at the edge of Swan Pool, Falmouth, England.
Slide no. 143/2/4 Nematology Dept., Rothamsted Experimental Station, Harpenden.

Cuticle smooth 2 μ thick, subcuticle 2 μ thick. No lateral fields. Head subrounded, not offset, lips rather small. Amphid apertures small transverse slits at the level of the dorsal tooth apex. In *en face* view the lip region as in figure 1B. Lips six, rounded on the inner edge, with two rings of papillae; inner ring of eighteen (three on each lip arranged in a short arc) and outer ring of ten (one at the peripheral apex of each lip, and one placed sub-laterally on each of the sub-median lips). In this view amphid apertures appear as crescentic slits. At level of dorsal tooth apex stoma deeper than wide, and the sclerotized elements are thickened where they abut.

In lateral view stoma almost three times as long as broad. Tooth apex at about 20-28 % of the stoma length. Anterior edge of the tooth straight, and sloping slightly posteriorly from apex. Posterior margin of tooth gently arcuate and merging imperceptibly with the stoma wall. Stoma wall with faint transverse striae, dorsal wall slightly indented below tooth apex. Stoma walls inarched above the tooth apex. Posterior elements not clearly marked off from anterior ones.

Oesophagus slightly expanded and surrounding basal third of stoma, then narrowing a little to the nerve ring at one quarter of the oesophageal length, then expanding again to occupy five-ninths of the body width. Dorsal oesophageal gland duct occurs at half the oesophageal length, duct of one pair of subventral oesophageal glands occurs at three-quarters of the oesophagus length, and the other duct at one oesophagus width from the end. Oesophago-intestinal junction with a narrow transparent zone interpolated between base of oesophagus and sphincter muscle, and flanked laterally by three (?) transparent bodies. Sphincter muscle solid, rather disc-like with an obtusely rounded conical structure projecting into intestinal lumen from the posterior face of the sphincter. Sclerotized lining of the oesophagus constricted near base of oesophagus and becomes less heavily sclerotized. After passing through the transparent zone and into the sphincter muscle the sclerotized lining fans out and becomes funnel-shaped. The walls of the "funnel" bear a number of plications. (The actual shape of this structure varies with the state of contraction of the muscle fibres). The lightly sclerotized "funnel" and the sphincter muscle form a valve between the oesophagus and the in-

testine (Figure 1D). Intestine a simple tube with abundant intra-cellular granules. In the female it passes to the right of the anterior and to the left of the posterior ovary. No pre-rectum in the female. Rectum straight, length equal to anal body width.

Vulva a transverse slit, one sixth body-width; lips oval, very feeble. Vagina feebly muscular, extending across less than one third of body-width; its lumen cruciform. Genital organs paired, opposed, ovaries reflexed through half the length of the uterus and oviduct. Up to four ova in the uteri at a time.

Tail conoid at first, then tapering more gradually to become almost cylindrical in the posterior two-thirds; tip rounded and slightly swollen. Three large caudal glands present, duct terminal. Paired lateral cuticular pores usually as shown in figure 1H.

Male (from DE MAN, 1884) "Schwanz des Männchens *von gleicher Gestalt* wie des Weibchens. Spicula *sehr schlank,* dünn, in eine sehr stumpfe Ecke gebogen, mit zentralem Verdickungsstreife, von zwei *ebenso schlanken,* aber viel kürzeren akzessorischen Stücken begleitet. Cuticula beim Männchen vor After schräg gestreift; 20 Papillen, nahe beieinander gelegen und kaum hervorragend, *vor* dem After und drei mediane ventrale wie auch einige laterale Papillen auf dem eigenlichen Schwanze." L = 1.9 mm.

REMARKS

M. truncatus and *M. macrostoma* are probably the same species. Apart from the supposed absence of cephalic papillae in *M. truncatus*, which is either an artifact or an oversight, the other differences mentioned by Bastian are unimportant. ANDRASSY (1958) has drawn attention to the variability of this species, with which he synonymised poorly known species such as *M. dadayi* Micoletzky, 1914, and *M. obtusus* Cobb, 1917. The six small tooth-like, cuticular, circum-oral spines mentioned by COBB (1917) in *M. dadayi* are the crudely drawn papillae. Daday's figures and descriptions are inadequate for identification. Butschli's figures of *M. obtusus* (copied by Cobb) are based on a partially distorted head which has been badly drawn. The stoma shown in these figures is of a different shape from that of *M. truncatus* and with the tooth apex at 39 per cent of the stoma length. The tail has a different shape, and the papillae are quite different. The status of Cobb's *M. longicaudatus* and *M. megalaimus* is not easily decided. *M. longicaudatus* is very similar to *M. truncatus*. These two species differ mainly in that males of the former have sixteen supplements compared with twenty in the latter, but as the range of variation of this character is not known, this criterion is of doubtful value for their separation. When Cobb erected *M. longicaudatus* in 1893 he does not appear to have seen *M. truncatus* (or *M. macrostoma*) from Europe. *M. longicaudatus* and *M. truncatus* are best regarded as synonyms until they can be compared. *M. megalaimus* is strikingly different from *M. truncatus* and *M. longicaudatus* in the feeble development of the lips and the close approximation of the anterior end of the stoma to the lips. Cobb's figure is not strictly lateral

so comparison of the teeth and stoma shape is not possible. *M. megalaimus* stands until the American mononchs are revised and its status determined. I regard *M. dadayi* Micol. and *M. obtusus* Cobb as *species inquirendae*.

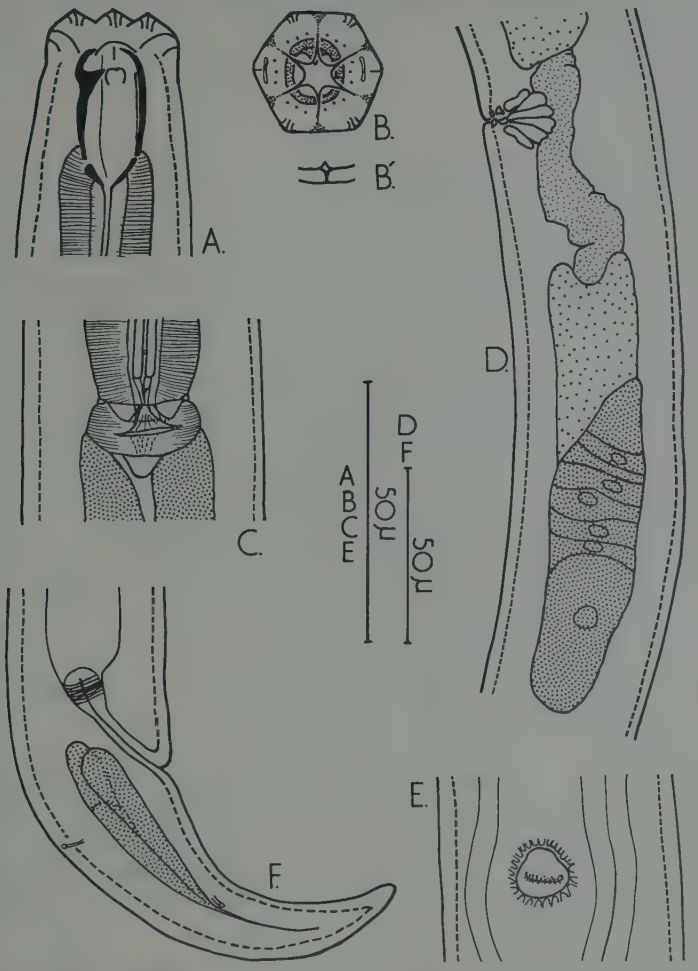

Figure 2. *Mononchus papillatus*. A. Head region; B. *en face* view of head (stoma cross section at the level of the dorsal tooth apex); B'. Enlarged view of ventral part of stoma showing ventral ridge in section; C. oesophago-intestinal junction; D. posterior half of female genital organs; E. ventral view of the vulva; F. female tail.

Mononchus papillatus Bastian
Figures 2A-F

Mononchus papillatus Bastian, 1865; p. 101, pl. IX, figs. 27, 28.
Mononchus (Mononchus) papillatus Cobb, 1916; p. 195.

Iotonchus papillatus Altherr, 1950; p. 4.
Prionchulus papillatus Altherr, 1952; p. 9.
Mononchus papillatus Andrassy, 1958; p. 1865.
nec Mononchus papillatus etc. in Butschli; 1873; Ditlevsen, 1911; Brakenhoff, 1914; Menzel, 1914; Hoffmanner & Menzel, 1915; Micoletzky, 1922 (partim); Micoletzky, 1925; Allgén, 1929; Altherr, 1938; de Coninck, 1939; Schuurmans Stekhoven & Teunissen, 1938; Schuurmans Stekhoven & Mawson, 1954.
nec Mononchus bastiani de Man, 1876.

Broadmoor.

Neotype ♀ L = 1.29 mm; a = 29; b = 3.9; c = 19; V = ^{16}62.2 ^{13}
♀♀ (n = 9) L = 1.23 mm; (s = ± 0.292 mm) (range 1.01-1.30 mm);
a = 22-29; b = 3.6-3.9; c = 14-19; V = $^{8-16}$60-67.7 $^{9-14}$

Neotype ♀ from turf, Broadmoor Institution, Berks. England. (Type locality).

Slide no. 143/1/7 Nematology Dept., Rothamsted Experimental Station, Harpenden, England.

Males extremely rare; only description THORNE (1924).

Cuticle smooth 1.7 μ thick; subcuticle smooth 1.7 μ thick. No lateral fields. Lip region scarcely offset, cephalic lips and papillae conspicuous in fresh material, less so in preserved specimens. Lips six, equal, symmetrical, inner margin rounded, outer margin obtusely angular. In *en face* view the papillae are readily seen in two rings, the inner ring of eighteen (three on each lip arranged in an almost straight line across the lip,) and an outer ring of fourteen (one at the apex of each lip and a sub-lateral pair on each of the sub-median lips). The innervations of the three inner papillae are distinguishable in lateral view, but usually only one papilla in the outer ring on the sub-median lips is visible in this view. Amphid apertures appear as crescentic slits in *en face* view. Stoma squarish in cross section at level of dorsal tooth apex. In this view dorsal tooth appears triangular in cross section, spreading slightly onto the dorsal wall. Opposite the dorsal tooth apex the abutting edges of the anterior subventral components of the stoma are thickened on the inner face. (This thickening forms the ventral ridge seen in lateral view). In lateral view the amphid apertures are difficult to see, but are situated at the level of the dorsal tooth apex. Viewed laterally the sclerotized stoma is cup-shaped, half as wide as long, and half as wide as the head width. Dorsal tooth apex occurs at 15-21 % of the stoma length. Anterior margin of tooth straight, horizontal or nearly so; posterior edge merges smoothly into stoma wall without any re-entrant angle. Dorsal wall of stoma indented a little posterior to tooth apex. On the ventral wall the tickened, abutting edges of the antero-ventral elements of the stoma form a low, untoothed ridge extending from a point opposite the dorsal tooth apex to the posterior limit of the anterior elements. Anterior to the tooth apex and ventral ridge, the stoma walls arch inwardly and restrict the size of the anterior aperture to about a third of the greatest width of stoma. Suture between anterior and posterior elements obscure. Postero-dorsal element much thickened anteriorly, but thins rapidly towards oesophageal lumen. Anterior three-

quarters of stoma surrounded by thin connective tissue sheath; posterior quarter embedded in anterior part of oesophagus.

Oesophagus muscular and glandular, narrowing slightly from stoma base to nerve ring at a quarter of oesophageal length, then expanding with general body contour to occupy slightly more than half the body width. Dorsal oesophageal gland duct opens into oesophageal lumen at mid point; one sub-ventral gland duct opens at two-thirds of its length; remaining duct opens half an oesophagus-width from end. Oesophago-intestinal junction more complex than existing figures indicate. Base of the oesophagus sub-rounded; from its posterior face an annulus of transparent tissue is surrounded by the sphincter muscle. Sclerotized lining of oesophagus becomes much thinner near base of the oesophagus; constricted where it passes through posterior end and starts to expand again as it passes through the transparent annulus, becoming funnel-shaped within the sphincter muscle. Attached to the posterior face of sphincter is a small conical projection, the shape and size of which varies from specimen to specimen. On either side of the oesophagus base and anterior to the sphincter muscle are small discrete transparent bodies. In life the thin sclerotized funnel is constricted by the sphincter muscle, and the whole forms a valve. Food passes rapidly through this valve.

Intestine a simple tube with abundant dark granules in its cells, passing to the right of the anterior and to the left of the posterior ovary. No pre-rectum in the female; rectum straight, length almost equal to the anal body-width.

Vulva a transverse slit one fifth body-width, with slightly thickened oval lips. Vagina muscular, extending across one third of body-width, and uniting with paired uteri. Genital organs paired, opposed, ovary reflexed through half to two-thirds the length of oviduct and uterus. Ovary half as wide as body, oocytes in a single row. No sphincter muscle or glandular region observed at the oviduct-uterus junction. Usually only one mature egg in the uteri; occasionally one in each uterus.

Tail conoid, ventrally arcuate, short. Three caudal glands present but no terminal duct; two pairs of lateral cuticular pores seen.

Differential diagnosis

Mononchus papillatus Bastian is most frequently confused with *Prionchulus muscorum* (Dujardin). For differentiation of these two species see page 192.

REMARKS

Several species may be hidden under the name *M. papillatus*. It is almost certain that the larva assigned to this species by SCHUURMANS STEKHOVEN & MAWSON (1954) from Soltanbad, Iran (L = 2.5 mm) does not belong here. The *M. papillatus* recorded from New Zealand and the Campbell Islands by ALLGÉN (1929) are probably *P. muscorum* (Dujardin), though rather small (L = 1.45 mm). The smallest specimen of this species I have from New Zealand is 1.68 mm long. The size of Mononchids varies considerably from habitat to habitat and 1.45 mm may be written within the range of variation for Campbell Island populations. It

is uncertain whether Allgén's dimensions relate to a specimen from New Zealand or Campbell Island.

M. bastiani de Man, 1876 has often been synonymised with *M. papillatus* but according to P. A. A. Loof (*in litt.*) de Man's specimen possesses the denticulate ridges of *P. muscorum* and is a synonym of that species.

M. cristatus Bastian, 1865 was synonymised with *M. papillatus* by Andrássy (1958). Bastian's description is brief and the figure (only specimen) has an obvious post-mortem artefact on the tail. The dimensions given are for an "immature female" for which L = 1.1 mm but Franklin (1955) has shown that for *Aphelenchus* spp. Bastians' measurements are 25 per cent too large. This holds for at least some of his mononchs. The corrected length of *M. cristatus* is 0.82 mm which is very small for *papillatus*; the value (c = 6) for *cristatus* indicates a tail two to three times as long as that found in *papillatus*. *M. cristatus* has not been recorded since it was described, and could not be recognised from the data available. I therefore regard it as a *species inquirenda.*

Over the years the names *Mononchus papillatus* and *Prionchulus muscorum* have been used about equally for species with denticulate and non-denticulate ridges. The confusion is well known. Dujardin stated that denticulate ridges were present in *muscorum,* but Bastian stated of *papillatus* "tooth single," and did not figure any ridge on the ventral stoma wall. Dujardin gave the length of *muscorum* as 2.56 mm, and Bastian's *papillatus* was $^{1}/_{11}$th in. (= 2.31 mm). Bastian's measurements are often 25 per cent too large (see above); this appears to hold for *M. macrostoma* and also appears likely for *M. papillatus*. If applicable, this reduces the length to 1.73 mm which is one fourth larger than any of my specimens from Broadmoor. This difference is not great for mononchids some of which (e.g. *Iotonchus parazschokkei* (Allgen), *M. propapillatus, Cobbonchus pounamua* n. spp. Clark in press) vary by a factor greater than two. No definite conclusions can be drawn from measurements, nor from Bastian's figure of the head which is distorted. One can only speculate whether Bastian overlooked a denticulate or a non-denticulate ridge. We may note that he knew of Dujardin's species as he reproduced the description. In the absence of any type specimens it is impossible to prove synonymity, or that two distinct species are indicated. Because both names have long standing I prefer to restrict them as Cobb, 1917 and T. Goodey, 1942, 1951 have done, using *M. papillatus* for the species with a non-denticulate ridge, and *P. muscorum* for the species with two denticulate ventral ridges.

Prionchulus Cobb, 1916

Mononchidae. Stoma large, almost parallel sided, armed with a single, stout, anteriorly directed, dorsal tooth situated in the anterior half, and with two subventral, denticulated, longitudinal, sclerotized ridges (only one visible in lateral view). Number of denticles variable, usually fewer and less well developed in larvae. Where the denticles are few they tend to be aggregated at the anterior

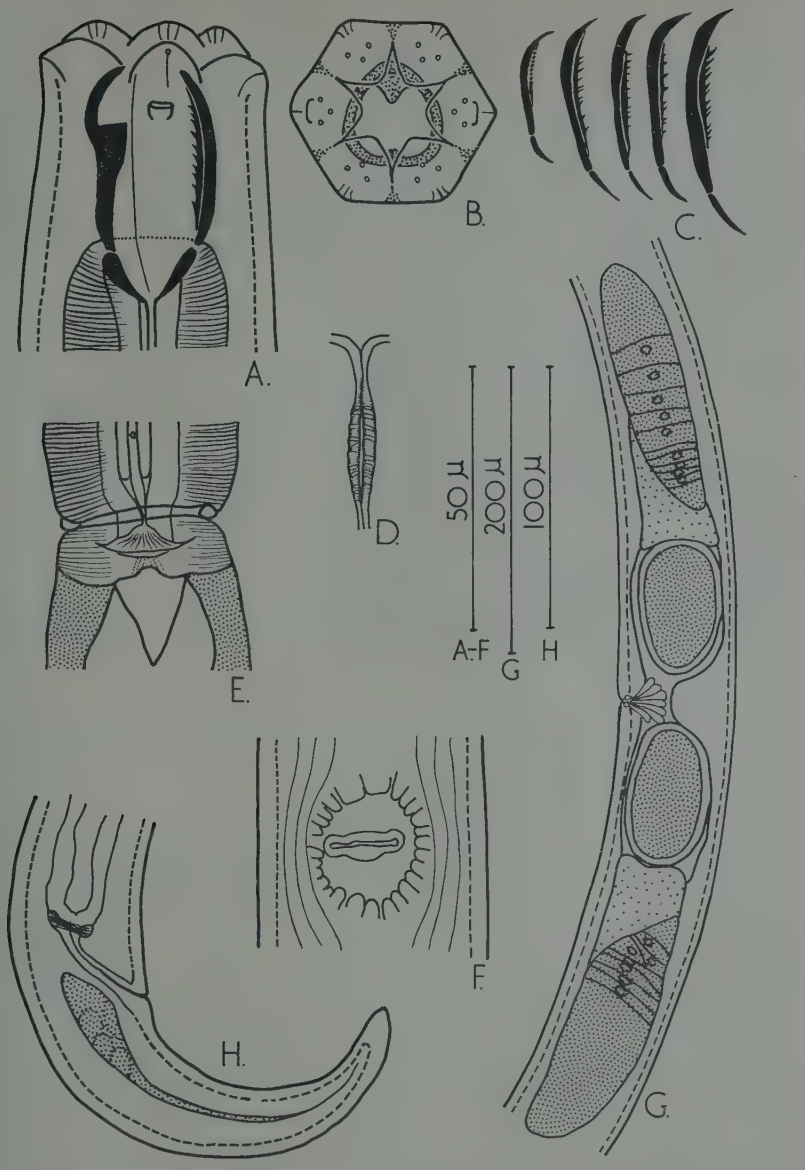

Figure 3. *Prionchulus muscorum*. A. Head region; B. *en face* view of head; cross section of stoma at the level of the dorsal tooth apex; C. lateral views of the denticulated ridges of some larvae showing variation in denticulation; D. ventral denticulated ridges viewed from the dorsal side; E. oesophago-intestinal junction; F. ventral view of the vulva; G. lateral view of female genital organs; H. female tail.

end of the ridges. Oesophago-intestinal valve non-tuberculate (CLARK, 1960); i.e. with stout sphincter muscle partially lined with lightly sclerotized, funnel-shaped extension of the oesophageal lining; never with sclerotized knobs or tubercles. Female genital organs paired, opposed, ovaries reflexed. Vulva 47-67 %. Spicules stout, arcuate; gubernaculum simple, but in all known males is associated with bifid lateral pieces. Tail usually short and conoid, rarely long and slender. Caudal glands present, no terminal duct. Paired lateral cuticular pores on tail (in males of *P. longus* Thorne these pores may be situated on papillae). Males rare or common. Found in soil, moss, leaf mould, and in sulphur springs (*P. thiocrenobius* Pax and Soós).

Type species: *Oncholaimus muscorum* Dujardin, 1845

Prionchulus muscorum (Dujardin, 1845) Chitw. & Chitw., 1937

Figures 3A-H

Oncholaimus muscorum Dujardin, 1845; p. 237.
Mononchus muscorum Bastian, 1865; p. 103.
Mononchus bastiani de Man, 1876; p. 184, pl. XIII, fig. 49a, b.
Mononchus (Prionchulus) muscorum Cobb, 1916; p. 195.
Mononchus (Prionchulus) muscorum macrolaimus Cobb, 1917; p. 163, fig. 38.
Prionchulus muscorum Chitwood and Chitwood 1937; p. 517.
Mononchus papillatus in Bütschli, 1873; Ditlevsen, 1911; Menzel, 1914; Hoffmänner & Menzel, 1915; Micoletzky, 1922 (partim); Micoletzky, 1925; Schneider, 1929 (partim); Allgén. 1929; de Coninck, 1939.

Broadmoor

♀♀ (n = 21) L = 2.12 mm; (s = ± 0.120 mm) (range = 1.91-2.32 mm)
a = 26-33; b = 3.3-4.4; c = 13-17;
V = $^{7-9}$ 61.3-66.7 $^{7-22}$

Cuticle smooth, 2.5 μ thick, subcuticle 1.5 μ thick. No lateral fields. Lip region not markedly offset. Lips and cephalic papillae readily distinguishable in lateral view (papillae less prominent in fixed material). Lip region hexagonal in *en face* view; lips six, equal, rounded on inner edge, obtusely angled on peripheral edge. Cephalic papillae in two rings, inner one of eighteen (three on each lip arranged in form of an inwardly directed obtuse triangle) and outer ring of fourteen (one placed at the apex of each lip, and a pair placed slightly laterally to this on each of the sub-median lips). Amphid apertures crescentic slits below the level of the lips. In this view the tripartite nature of the stoma is clearly seen. At the level of the dorsal tooth apex stoma rather triangular in cross section. Along the ventral suture the ventro-lateral elements are slightly invaginated and thickened on the inner surface where they abut. Transverse thickenings along these ridges form the denticulations characteristic of the genus. Dorsal tooth triangular in cross section spreading considerably onto the dorsal wall of the stoma.

In lateral view the inner ring of papillae is easily distinguished, but, of the outer ring, only the apical papillae are easily seen. In lateral view pouch-like amphids

are situated between the tooth apex and the anterior limit of the sclerotized stoma. Stoma twice as long as broad, cup-shaped with pointed base. Dorsal tooth strong, with apex at 25-30 % of stoma length. Anterior edge of the tooth straight, almost horizontal or sloping slightly posteriorly from the apex. Posterior or buttress edge merging imperceptibly with the dorsal stoma wall. On the ventral wall of the stoma lie the denticulate ridges bearing nine to fourteen denticles in the mature female. In larvae these denticles are irregular in development and number. Dorsal wall of stoma indented laterally to the tooth; both walls inarched above the tooth apex, and the anterior orifice of the stoma restricted to two-fifths of the greatest diameter of the stoma. Suture between the anterior and posterior stomal elements obscure. In lateral view the dorsal posterior element appears much thicker than the ventral ones, but becomes thinner towards the oesophageal lumen. Basal quarter of the stoma enveloped by the anterior end of the oesophagus. Oesophagus uniform in width to the nerve ring which occurs at a quarter of its length; then expanding a little with the general body contour and occupying three-fifths of the body width. Dorsal oesophageal gland duct opens into the oesophageal lumen at 60 per cent of the oesophagus length and the subventral gland ducts open at 80 and 95 per cent respectively.

Oesophago-intestinal junction with thin transparent zone interpolated between sphincter muscle and base of oesophagus. Flanked on either side by 3 (?) smaller transparent bodies. Sphincter stout and disc-like. A little before the end of the oesophagus its sclerotized lining becomes much thinner and is constricted as it passes through the oesophagus base and the transparent area. In the sphincter, this flares out again to form a shallow bell-like structure, much plicated in its mid-region. Its tripartite nature detectable only with difficulty. The shape of the sclerotized structure varies with the contraction of the sphincter muscle fibres. Posterior surface of sphincter bears a short conical structure the shape of which is rather variable, invisible in most specimens. Intestine a simple tube, cells with abundant dark granules. Intestine passes to the left of the anterior and to the right of the posterior ovary. No pre-rectum; rectum straight, equal to anal body-width.

Vulva a transverse slit, one-third as wide as the body, with broad, variable, slightly thickened lips. Vagina moderately muscular, extending inwards about a third of body width, and uniting with the uteri. Genital organs paired, opposed, ovaries reflexed through a third to half the length of oviducts and uteri. Oocytes arranged in a single row. No sphincter or glandular region between the oviducts and uteri. Length of the uteri varies with the number of mature eggs contained. Egg shells may be ridged. Often only one mature egg in each uterus, but there may be two in each.

Tail short, conoid, ventrally arcuate. Caudal glands present, but no spinneret. Two pairs of lateral cuticular pores as shown in figure 2F.

Male (after SCHNEIDER, 1923)

n = 2 L = 2.16-2.24 mm; a = 36-37; b = 4.0-4.14; c = 18-18.7.

Supplements sixteen evenly spaced, the first, three tail lengths from the anus. Spicules paired, large, arcuate. Gubernaculum small, partially enfolding the spicules. Notched lateral accessory pieces present. Tail similar to female.

REMARKS

One adult female *P. muscorum* from Broadmoor appeared to be undergoing an adult moult. The specimen had the normal, fully developed genital structures, a patent vulva in the cuticle that was being shed, and another lying beneath it in the newly formed cuticle. The stoma had two sclerotized linings; one in the process of being moulted. There were no mature eggs in the uteri, the oocytes in the ovaries were rather small, and the length of the eelworm was only 1.62 mm (smaller than any other females I have measured) and therefore probably precociously mature, rather than undergoing an additional moult.

P. muscorum appears to be a cosmopolitan species. Because the figures of *Mononchus muscorum* of Schuurmans Stekhoven & Teunissen (1938) lie quite outside the range of variability encountered so far this species, the specimens from which these figures were drawn are believed to be a new and undescribed species. No measurements were given, and the males were not described or figured.

Andrássy (1958) provides a synonymy for *P. muscorum* but gives no reasons for including some of the forms. *M. papillatus* forma *macrodon* Fuchs is based on a specimen of *papillatus* with a partially collapsed stoma. There are no grounds for placing it in this genus as denticulate subventral ridges are neither figured nor mentioned. He has overlooked the echinulate eggs of *P. punctatus* Cobb, a feature unknown in *muscorum*. The status of *M. (P.) longicollis* Cobb is in doubt and as it appears to be based on a single larva I regard it as a *species inquirenda*. *M. (P.) muscorum macrolaimus* Cobb is of doubtful status; there appear to be some differences between the two forms but until specimens can be compared I agree with Andrássy and regard it as a synonym of *P. muscorum*. The single male of *P. spectabilis* (Ditlevsen) described by Meyl (1955) does not agree with the characters of *spectabilis*; there are far too many denticles on the ventral ridges and the length is only 1.52 mm whereas Ditlevsen's males measured 3.6 mm. Meyl's specimen may be a male of *P. muscorum*, but until similar specimens are found associated with females it is best to regard *P. spectabilis* Meyl, 1955 *nec* Ditlevsen, 1911 as a *species inquirenda*.

CONCLUSION

Adults of *M. papillatus* and *P. muscorum* are readily distinguishable. Most of the confusion between them has arisen from attempted determinations of larvae, and probably from the intrusion of a third species, *P. punctatus* Cobb, 1917 (= *M. muscorum* of Brakenhoff, 1914), a species characterised by poorly developed, rather short denticulate ridges in the stoma, and by the presence of echinulate eggs. Echinulate eggs are not found in *muscorum*, but have been re-

corded by BRAKENHOFF (1914), and ALTHERR (1938). The figures of these authors are not of typical *muscorum* or *papillatus*. *M. echinulatus* Altherr, 1938 appears to be a *lapsus* for *M. punctatus*. Specimens of *P. punctatus* were not available for consideration.

The described species of *Prionchulus* are chiefly to be recognised from the characters set in Table I. Most species are imperfectly known, the range of variation being quite unknown, and probably many more species await description.

TABLE I

Characters of the described species of Prionchulus

species	♀ L.	c	tail	♂ L.	c	no. of denticle on ridges
muscorum (Dujardin)	1.9-3 mm	12.5-17	conoid short	—	—	9-14
longus (Thorne)	2.14 mm	20	conoid short	2.0 mm	27	20
punctatus Cobb	1.7 mm (echinulate eggs)	14.3	conoid short	—	—	12 (ridges short)
spectabilis (Ditl.)	4.0 mm	8	conoid short	3.6 mm	?	7
thiocrenobius Pax & Soos	1.6 mm	5.5	longish slender tip swollen	— (V = 47 %)	—	16-17 (?) (ridges short)

M. papillatus and *P. muscorum* may be readily separated as follows:

M. papillatus	*P. muscorum*
Ventral ridge low, never denticulate	Ventral ridges prominent, always denticulate; denticulations variable in larvae.
Inner papillae on each lip arranged in a straight line	Inner papillae on each lip arranged in an obtuse triangle.
Stoma squarish in cross section at the level of dorsal tooth apex	Stoma rather triangular in cross section at level of dorsal tooth apex.
Ventral ridge terminates at level of tooth apex	Ventral ridges extend a little anterior to dorsal tooth apex.
Not more than two eggs in uteri at a time	Up to four eggs in uteri at a time.
L = 1.0-1.36 mm (British specimens)	L = 1.91-2.64 mm (British specimens).
A soil dwelling species	Predominantly a moss species.

ZUSAMMENFASSUNG

Neubeschreibung von Mononchus truncatus *Bastian*, M. papillatus *Bastian und* Prionchulus muscorum *(Dujardin)* (*Enoplida, Nematoda*)

Mononchus truncatus Bastian (= *M. macrostoma* Bastian) und *M. papillatus* Bastian werden aus topotypischem Material neubeschrieben und Enotypen bestimmt. *Prionchulus muscorum* (Dujardin 1845) Cobb 1916 wurde aus britischen Populationen neubeschrieben. Die Unterschiede von *M. papillatus* und *P. muscorum* sind folgende:

M. papillatus	*P. muscorum*
Ventrale Leiste niedrig, nie gezähnt.	Ventrale Leisten hervorstehend, stets gezähnt, Zähnelung unterschiedlich bei Larven.
Innere Papillen an jeder Lippe in einer geraden Linie angeordnet.	Innere Papillen an jeder Lippe zu einem stumpfen Dreieck angeordnet.
Mund im Querschnitt fast quadratisch in Höhe der dorsalen Zahnspitze.	Mund im Querschnitt mehr dreieckig im Höhe der dorsalen Zahnspitze.
Ventrale Leiste endet auf der Höhe der dorsalen Zahnspitze.	Ventrale Leisten erstrecken sich ein wenig nach vorn zur dorsalen Zahnspitze.
Im Uterus nicht mehr als 2 Eier gleichzeitig.	Im Uterus bis zu 4 Eier gleichzeitig.
L = 1,0-1,36 mm (Britische Proben).	L = 1,91-2,64 mm (Britische Proben).
Bodenbewohnende Art.	Vorwiegend eine moosbewohnende Art.

P. punctatus Cobb 1917 kann die Bestimmung beider Arten erschweren. *M. dadayi* Micoletzky 1914, *M. obtusus* Cobb 1917, *M. cristatus* Bastian 1865 und *P. longicollis* Cobb 1917 werden als species inquirendae angesehen. *Mononchus echinatus* Altherr 1938 ist ein Lapsus für *P. punctatus* Cobb.

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NEW ZEALAND MONONCHIDAE (ENOPLIDA, NEMATODA)
I THE GENERA *MONONCHUS* BASTIAN AND *PRIONCHULUS* COBB

BY

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Free-living nematodes of the New Zealand region have been little studied, the only important papers being those of COBB (1904), and ALLGÉN (1929). Cobb described *Miconchus rex* (Cobb, 1904) Andrássy, 1958 from 200-1200 feet in Lakes Manapouri and Wakatipu, and Allgén recorded *"Mononchus papillatus"* Bastian = *Prionchulus muscorum* (Dujardin) in moss from New Zealand and Campbell Island, and *Iotonchus trichurus* Cobb, *I. parazschokkei* (Allgén), and *Mononchus campbelli* Allgén in moss from Campbell Island. The determination of *I. trichurus* from a single larva is doubtful.

The material described in this and succeeding papers was collected by the author, killed by heat, fixed in F.A. 4 : 10, and transferred to glycerine by Baker's method.

Mononchus Bastian, 1865

Mononchus campbelli Allgén, 1929

Allgén's inadequate description is based on two poorly fixed distorted larvae from moss on Campbell Island. No precise locality is given, and no type specimens appear to have been kept. ANDRÁSSY (1958) synonymised *M. campbelli* with *"M. papillatus"* of SCHUURMANS STEKHOVEN & TEUNISSEN (1938) from the Belgian Congo, and tentatively with *M. allgéni* Meyl, 1957 from Lake Tanganyika. However, c for *M. campbelli* is 7.2 against 12.9 for *M. allgéni*. In *"M. papillatus"* c = 16 for larvae and 11.5 for adults, the dorsal tooth is situated more anteriorly in the stoma and the tail shape differs. Zoogeographically it is unlikely that the same species would occur in the Belgian Congo and on a subantarctic island. No useful purpose is served retaining this doubtful species and I regard *M. campbelli* as a *species inquirenda*.

Mononchus composticola n. sp.

Figs. 1A-J

Holotype ♀ L = 2.44 mm; a = 33; b = 4.67; c = 9.2; V $^{11}50^{12}$
Allotype ♂ L = 3.42 mm; a = 39; b = 4.75; c = 21.5; T = 53.

For measurements of paratypes see Table I.

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[1]) At present on study leave from Entomology Division, D.S.I.R., Nelson, New Zealand.

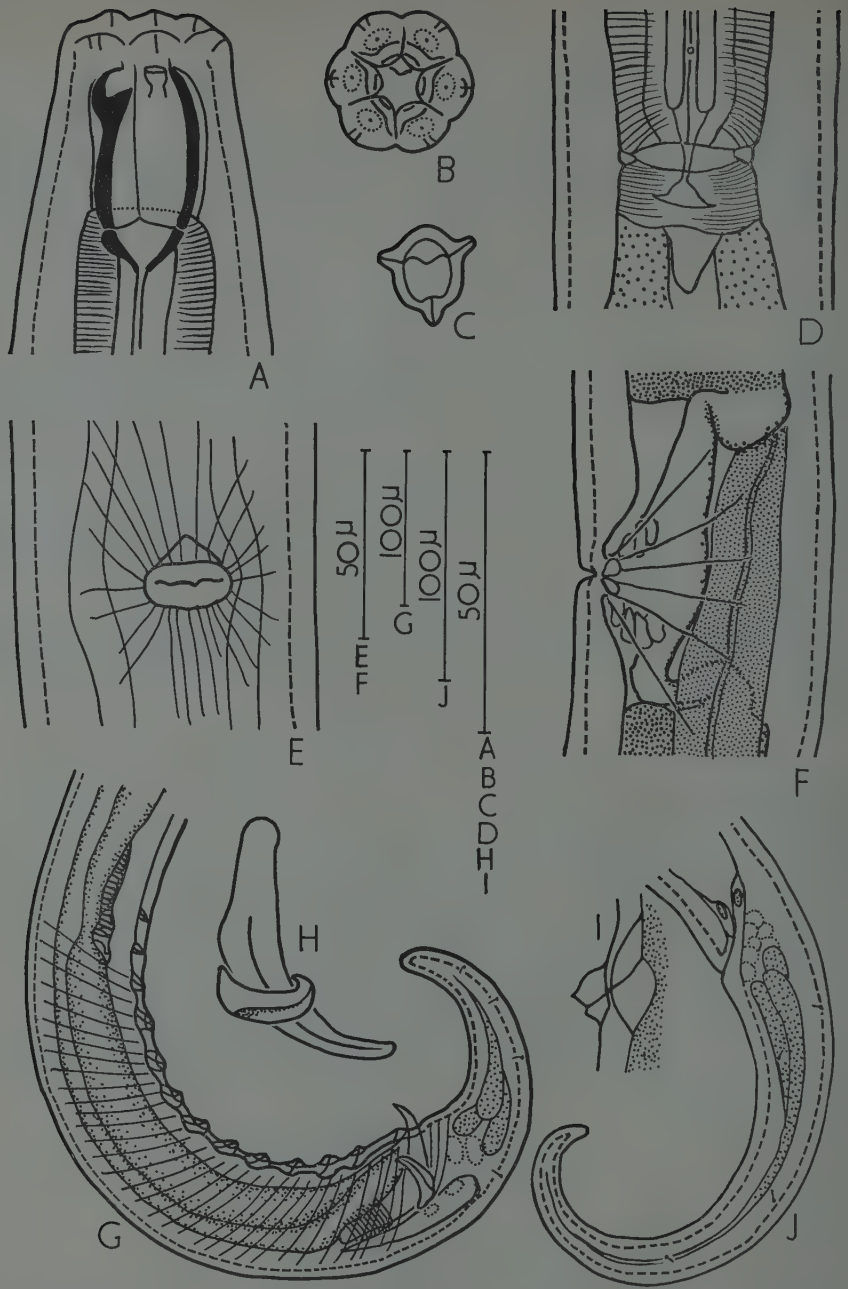

Fig. 1. *Mononchus composticola* n. sp. A. lateral view of head, B. *en face* view of lip region, C. stoma at level of tooth apex, D. oesophago-intestinal junction, E. ventral view of vulva, F. lateral view of vulva, G. male tail, H. spicules and gubernaculum, I. supplement, J. female tail.

TABLE I

Measurements of *Mononchus composticola* n. sp.

	Mt. Cook	The Chateau	Tutamoe	Pelorus Br.	Waiotapu	Arthurs Pass (females)	Arthurs Pass (males)
n.	7	10	12	1	7	36	3
.. (mm) mean)	2.18	2.08	2.17	2.59	2.35	2.33	3.22
.D. mm	± 0.323	± 0.124	± 0.169	—	± 0.192	± 0.166	—
ange	1.76-2.66	1.88-2.29	1.91-2.44	2.59	2.11-2.60	1.83-2.70	3.04-3.51
	27-38	25-38	33-42	31	30-35	27-36	35-38
	4.21-5.25	3.9-4.5	3.68-5.05	5.18	4.6-5.0	3.76-4.95	4.5-4.7
	7.5-10.9	7.2-8.4	7.4-9.8	11.0	8.5-10.7	7.2-9.9	17.8-22.0
.o. 1)	7-9	10-13	9-14	8	8-11	7-9	—
7	45.4-54.2	50.8-54.7	48.5-54.7	47.7	44.8-48.9	48.3-52.0	T. 52.4-57.9
.o. 1)	7-13	8-12	10-13	12	7-10	5-13	—

1) Anterior and posterior ovaries respectively.

Measured specimens from Levin Bush, Hongi's Track, Takaka Hill, and Lake Mahinapua all fell within the range of measurements given above.

Cuticle 2.5 μ thick, with fine, faint annulations only visible under oil immersion in favourable circumstances. No lateral fields. Subcuticle 2.0 μ thick. Body outline tapering anteriorly; lip region not offset. Lips and papillae clairly visible in lateral view. Amphid apertures elliptical in lateral view, situated a little anterior to the apex of the dorsal tooth. Amphid constricted to two-thirds aperture width at a distance equal to aperture width, then expanding again.

In *en face* view lip region a rounded hexagon, symmetrical, all six lips rounded on both edges. Papillae in two rings; six in the inner ring and ten in the outer ring, (two on the sub-ventral lips and one on each lateral lip). Amphid apertures appear as crescentic slits in this view. The abutting edges of the anterior elements of the stoma skeleton elements appear thickened, project outwards, and are coincident with the clefts between the subdorsal and lateral lips, and between the subventral lips. At the level of tooth apex the tooth spreads onto the whole of the dorsal stoma wall. In lateral view the rather small stoma occupies half the body width, is attenuated oval in shape, more pointed posteriorly than anteriorly. Anterior aperture approximately half the greatest stoma width. Dorsal wall bears a single strong tooth the anterior edge of which is straight or slightly curved, and slopes slightly back from apex. Tooth apex at 6-17 per cent of stoma length. The posterior edge or buttress of the tooth is continuous with the contour of the posterior part of the wall. Whole tooth lies within anterior two-fifths of stoma. Anterior to the tooth the dorsal wall curves inwards. Outer surface of stoma wall indented in region of tooth. Anterior and posterior ventral elements curve smoothly throughout their length. Posterior dorsal element much thickened in anterior half, but thinning towards oesophageal lumen. Anterior two-thirds of stoma sur-

rounded by a thin muscle sheath; posterior third embedded in anterior end of the oesophagus.

Oesophagus slightly expanded anteriorly about stoma base; narrowing a little to nerve ring at one third of oesophageal length; expanding slightly beyond this in conformity with the body contour, and occupying half the body width.

Oesophago-intestinal junction non-tuberculate, (CLARK, 1960a) with a strong sphincter muscle enveloping the funnel-shaped valve. A fairly broad transparent zone is intercalated between oesophagus and sphincter muscle. A conical structure projects into intestinal lumen. Intestine is a simple tube; cells packed with fine granules.

Female. Vulva a transverse slit one quarter body-width, with thickened lips. Radiating muscle bands are conspicuous in ventral view. Vagina thick-walled and extending across half the body-width. Uterus bifid; size varies with stage of oögenesis. Ovaries paired, opposed, reflexed through two thirds of the length of the oviduct and uterus. Ovary occupying up to four-fifths of body width. Oöcytes in a single row. Usually one or two mature eggs at a time; rarely three. Intestine passes to the left of the anterior and to the right of the posterior ovary. Pre-rectum not demarcated in the female. Rectum straight, length almost equal to an anal-body-width.

Tail moderately elongate (five and a half to six times anal-body-width in length), conoid, ventrally arcuate, tip rounded. Caudal glands present; terminal duct absent. Four pairs of lateral cuticular pores distributed as shown in figure 1.

Male. Testes paired, opposed, outstretched. Relative development of anterior and posterior testes variable. Sperms numerous, not arranged in any definite pattern. Testes nine-tenths as long as vas deferens. Opposite the second supplement the vas deferens enlarges to become the ejaculatory duct, and continues as such to its junction with the cloaca. Pre-rectum as long as ejaculatory duct.

Spicules separate, stout, arcuate, more slender at tips. Upper portions of spicules embedded in a muscular sheath. Gubernaculum broad, U-shaped, strap-like, almost encircling the spicules. Each arm of the U with a thickened supporting ridge running laterally from the posterior face near the base of the U to near the anterior edge of the tips of the lateral processes. Midventral supplements 12, not echinulate, mostly mammiform, but the mammiform protuberances not well developed at the ends of the series. An adanal pore is present. Anterior to the supplements are a number of simple cuticular pores.

Tail short, two and a half times anal-body-width, conoid, ventrally arcuate, tip rounded. Caudal glands present, terminal duct absent. Caudal pores as shown in figure 1 G.

Specific differentiation. M. composticola forms with *M. major* Cobb, 1893, *M. mesadenus* n. sp., and *M. gerlachei* de Man, 1904 a closely related group of southern species. The only similar European species is *M. kastrolli* Altherr, 1958 which is readily separated from this species-group by the short tail (c = 33 or more), the presence of bifid spicules, and notched lateral accessory pieces in the

male. A new figure of the spicules etc. of *M. kastrolli* (drawn from paratypes supplied by Dr. Altherr) is provided (fig. 2). Of the New Zealand species, *M. composticola* is similar to *M. mesadenus* from which it is separated by the more slender female tail; c is usually within the range of 7-9.5 (*mesadenus* c = 10-16.5) and the presence of vulval gland ducts opening through the cuticle anterior and posterior to the vulva in *mesadenus,* but not in *composticola.* Males may be

Fif. 2. *Mononchus kastrolli* Altherr, spicule, gubernaculum and lateral accessory piece.

distinguished by the supplements which number twelve in *composticola*; nine in *mesadenus,* and are echinulate only in the latter.

Mononchus composticola, major, mesadenus and *gerlachei* may be separated by the use of the following keys:

Key to males:

1. Lateral accessory pieces rounded at tip — *M. gerlachei*
   Lateral accessory pieces absent 2
2. Supplements echinulate 3
   Supplements not echinulate, twelve — *M. composticola*
3. Nine supplements; sixteen cephalic papillae — *M. mesadenus*
   Twelve supplements; thirty-two cephalic papillae — *M. major*

Key to females:

1. Glands present near vulva 2
   Glands not present near vulva — *M. composticola*
2. Terminal caudal gland duct present 3
   Terminal caudal gland duct absent — *M. mesadenus*
3. Sixteen cephalic papillae, (Danco Land and Possession Island, Antarctic region (de Man, 1904, Steiner, 1916)) — *M. gerlachei*
   Thirty-two cephalic papillae (Australia & Tasmania (Cobb 1893, 1917) — *M. major*

Type slides. Holotype, allotype and paratype slides in nematode collection, Entomology Division, D.S.I.R., N.Z. Paratypes also at Nematology Dept. Rothamsted Experimental Station. Slide nos. 143/4/1-2.

Type locality. Rough Creek, Arthurs Pass, N.Z., from leaf litter of *Nothofagus cliffortiodes* Oerst. forest with *Coprosma* Forst spp., *Nothopanax simplex* Seem., *Griselinia littoralis* Raoul, and *Blechnum pennamarina* Kuhn, altitude 765 metres; soil Haast fine sandy loam.

Other localities. (a) South Island; Takaka Hill in *Nothofagus fusca* Oerst. forest; Lake Mahinapua Forest Reserve in litter of *Quintinia acutifolia* T. Kirk — *Weinmannia racemosa* Linn. f. forest; Pelorus Bridge in litter of *Podocarpus spicatus* R. Br. — *Beilschmiedia Tawa* Benth. & Hook. f. forest; Governors Bush, Mt. Cook in *Nothofagus Menziesii* Oerst. forest litter. (b) North Island: Levin Bush in litter of *Knightia excelsa* R. Br. — *Beilschmiedia Tawa* Benth. & Hook. f. forest; Tongariro National Park, near Chateau, in *Nothofagus cliffortiodes* Oerst. — *Griselinia littoralis* Raoul forest litter; Tutamoe in litter of *Beilschmiedia Tawa* Benth. & Hook. f. — *Dacrydium cupressinum* Soland. forest.

Remarks. This species has been found only in the litter of indigenous forests in areas with a rainfall in excess of 40 in. per annum, though most localities approach more closely to 100 in. per annum. Neither the soil underlying the litter nor the species composition of the forest appear important in determining distribution. *M. composticola* may be equally abundant in small-leaved mor type, *Nothofagus* litter and in the broad-leaved, mull type *Beilschmiedia* forest. The association with forest litter suggested the specific epithet.

A parasitic sporozoan, *Legerella* sp. was found in a specimen from Arthurs Pass.

Mononchus mesadenus n. sp.

Figs. 3A-K.

Holotype ♀ L = 3.37 mm; a = 40; b = 4.45; c = 12.4; V = ^{11} 54 ^{10}
Allotype ♂ L = 3.26 mm; a = 40; b = 4.34; c = 20.5; T = 53.

For measurements of paratypes see Table II.

Cuticle 3.5 μ thick, smooth, very faint obsolescent transverse striae occasionally seen under oil immersion. Lateral fields not present. Subcuticle 2.5 μ thick, faintly annulated. Lip region not offset; lips and papillae clearly visible in lateral view. Amphid apertures appear circular in lateral view, are one sixth as wide as the lip region, and narrow rapidly. Amphids are situated near the anterior limit of the sclerotized stoma and immediately posterior to the lateral papilla. In *en face* view the lip region is bilaterally symmetrical, an elongate hexagon. Lips six, equal, inner edge bluntly rounded; outer edge slightly angled. Papillae in two rings, an inner ring of six and an outer ring of ten. Inner papillae flanked on either side by a rounded eminence. In this view the anterior edge of the stoma resembles that of *M. composticola,* but the dorsal tooth does not spread onto the stoma wall to the same extent. Laterally the stoma appears heavily sclerotized, twice as long as broad, oval in shape; fairly rounded posteriorly, and occupies just over half the body width. Anterior aperture half as wide as the greatest stoma width. Stoma walls with fine transverse striae. Dorsal tooth apex occurs at 7-16 % of the stoma length. Anterior edge of the tooth straight or slightly curved; sloping a little back

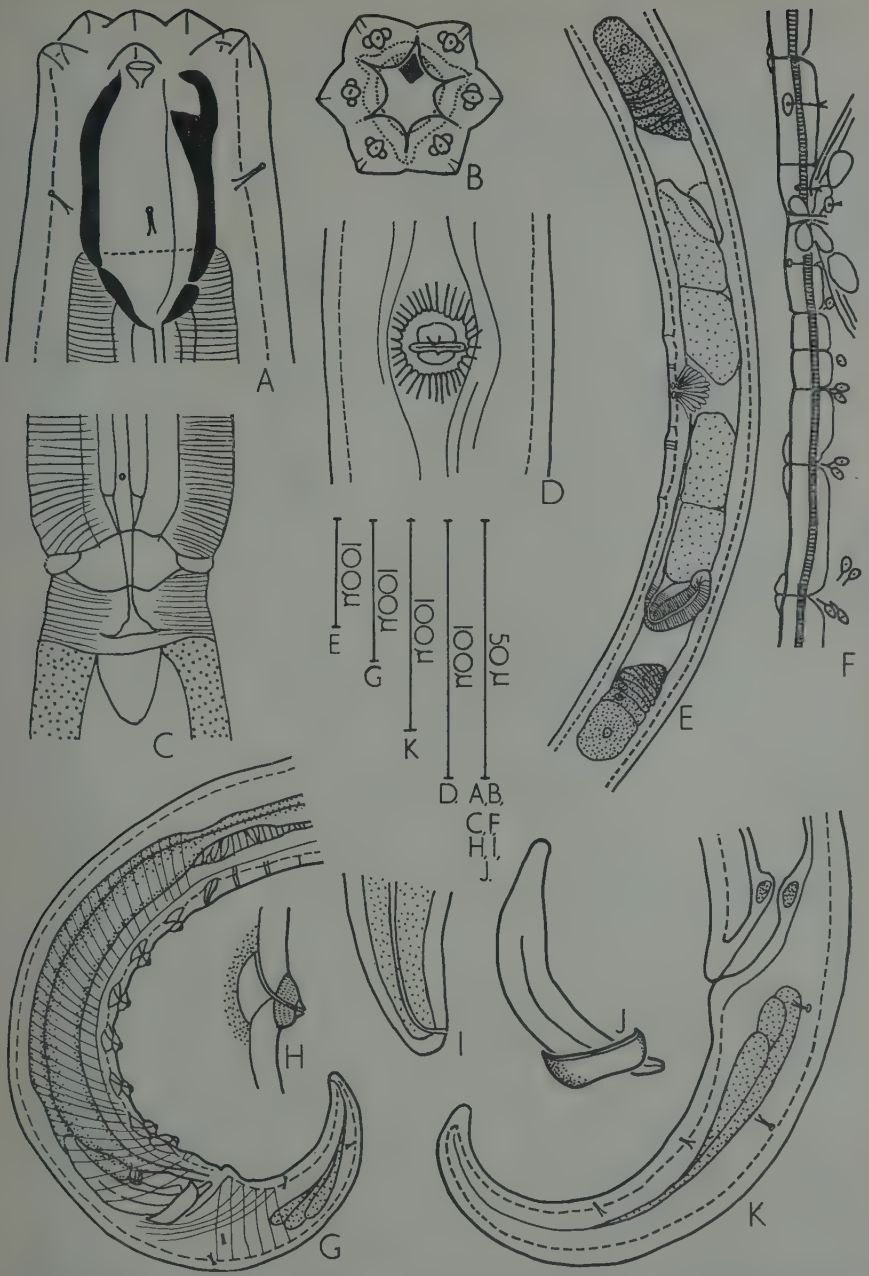

Fig. 3. *Mononchus mesadenus* n. sp. A. lateral view of head, B. *en face* view of lip region, C. oesophago-intestinal junction, D. ventral view of vulva, E. female reproductive organs, F. vulval region showing gland cells and ducts, G. male tail, H. supplement, I. tip of male tail, J. spicule and gubernaculum, K. female tail.

TABLE II

Measurements of *Mononchus mesadenus* n. sp.

Females	Mt. Messenger	The Chateau	Tutamoe	Kiripaka	Takaka	Hongi's Track
n	9	6	6	2	2	1
L (mm) (mean)	3.17	3.35	3.09	3.78	3.32	2.49
S.D. (mm)	± 0.183	± 0.227	± 0.128	—	—	—
range	2.87-3.44	3.08-3.72	2.94-3.26	3.77-3.79	3.28-3.36	2.49
a	35-40	35-38	32-39	38	35-39	33
b	3.99-6.09	4.10-4.72	4.26-4.45	4.91-4.93	4.56-4.82	4.89
c	10.5-13.1	10.1-13.1	10.2-12.6	13.8-13.9	13.6-16.4	15.6
a.o.	9-15	7-15	8-15	11-13	9-11	7
V	47.2-56.0	51.0-53.5	51.3-55.3	51.5-52.2	54.4-55.8	51.8
p.o.	8-13	7-13	8-16	11	12	9
Males	Mt. Messenger	The Chateau	Tutamoe	Kiripaka	Rimu	Collingwood
n	13	7	10	1	2	1
L (mm) (mean)	3.10	3.09	2.94	3.71	3.00	2.70
S.D. (mm)	± 0.185	± 0.225	± 0.177	—	—	—
range	2.61-3.35	2.94-3.62	2.65-3.20	3.71	2.78-3.23	2.70
a	31-40	35-40	34-41	39	32	36
b	4.15-4.55	3.96-4.26	3.99-4.45	4.71	4.02-4.03	3.88
c	16.0-20.8	15.0-18.1	14.6-20.1	22.3	17.0-18.3	22.3
T	53.1-57.2	41.4-62.4	38.8-48.2	58.7	40.1-54.8	53.2

from apex. Posterior edge slopes smoothly back and joins stoma wall without any re-entrant angle. Opposite the dorsal tooth the outer surface of the dorsal stoma wall is slightly indented. Anterior to the tooth the wall curves sharply inwards. Lower three-fifths of the ventral wall almost straight, anterior portion curving slightly inwards. Of the posterior elements the dorsal one is thickest anteriorly; both thin towards the oesophageal lumen. Anterior seven-tenths of stoma surrounded by a thin muscular sheath; posterior part embedded in anterior end of oesophagus. In the stomal region is a pair of subventral, subdorsal, and median cuticular pores.

Oesophagus narrows slightly to the nerve ring which occurs at one third of the oesophagus length, and thereafter swells a little generally occupying half the body width. Oesophago-intestinal junction non-tuberculate. Transparent zone between the oesophagus and sphincter muscle often wide (one-third as thick as sphincter muscle). Funnel-shaped valve obscure in all specimens. Intestine packed with dark granules.

Female. Vulva a transverse slit with thickened lips. Vagina with thick muscular walls, extending half way across the body. Uteri paired, outstretched. Interposed between uterus and oviduct is a thick-walled glandular structure and a sphincter muscle similar to that recorded by GOODEY (1942) in *Anatonchus tridentatus*

and *Mylonchulus brachyuris*. Ovaries paired, reflexed through up to one-third of the length of the oviduct and uterus. Ovaries occupying almost half the body width. One or two mature eggs in the uteri at once.

Near the vulva are a number of pores through the cuticle which appear to be connected with small gland cells. The largest of these have small cuticular mounds about the orifices of the ducts; the smaller ones do not. These structures tend to be arranged in ventro-median pairs, but exceptions are numerous. Some ducts are always present, but the number and position of both types of duct is variable. Simple body pores are present over the whole of the animal, but the papilliform pores are restricted to the vicinity of the vulva.

The intestine passes to the left of the anterior, and to the right of the posterior ovary. No pre-rectum. Rectum slightly arcuate, equal to anal-body-width.

Tail short, equal to four and a half times the anal-body-width; conoid, ventrally arcuate tip rounded. Caudal glands present; no terminal duct. Paired lateral cuticular pores present, often arranged as in figure 3 K, but there appears to be considerable variation.

Males. Testes paired, opposed, outstretched, sperms abundant, not arranged in any definite pattern. Vas deferens five-sixths of testicular length, dilating opposite the first supplement to become the ejaculatory duct and discharge into the cloaca. Also opposite the first supplement the intestine narrows to form the prerectum. Spicules separate, arcuate, thinning towards the tips with a median thickened rib in the distal two thirds. Total length of spicules equal to one and two-thirds anal-body-widths. Gubernaculum U-shaped, strap-like, almost encircling the spicules. Each arm of the U with a thickened anterior edge. Supplement nine, the middle seven mammiliform echinulate; first and anal supplements with low mounds rather than papillae. Anterior to the supplements is a series of body pores. In ventral view the basal echinulate portions of the supplements are seen to consist of two opposed crescentic swollen ridges.

Tail shorter than female; equal to two anal-body-widths, conoid, ventrally arcuate, with caudal glands and terminal duct. Paired lateral cuticular pores as shown in figure 3 G.

Specific differentiation. See under *M. composticola.*

Type material. Holotype, allotype, and paratype slides in the nematode collection of Entomology Division, D.S.I.R., N.Z. Paratypes also at Nematology Dept., Rothamsted Experimental Station. Slide nos. 143/5/1-2.

Type locality. Mt. Messenger, Taranaki, N.Z., N.Z.M.S. 1, N. 100, 146126, in *Olea Cunninghamii* Hook. f., *Vitex lucens* T. Kirk, *Shawia paniculata* Forst., *Brachyglottis repanda* Forst. forest litter; altitude 200 metres.

Other localities: (a) South Island: Collingwood from litter in a very damp spot in a *Rhopalostylis sapida* Wendl. & Drude grove; Takaka Hill, in litter of *Nothofagus fusca* Oerst. forest; Rimu State Forest (*Thuya plicata* Don. + regenerating natives) surface liverworts and mosses, (b) North Island: Tongariro National Park, near the Chateau in *Nothofagus cliffortiodes* Oerst. — *Griselinia littoralis*

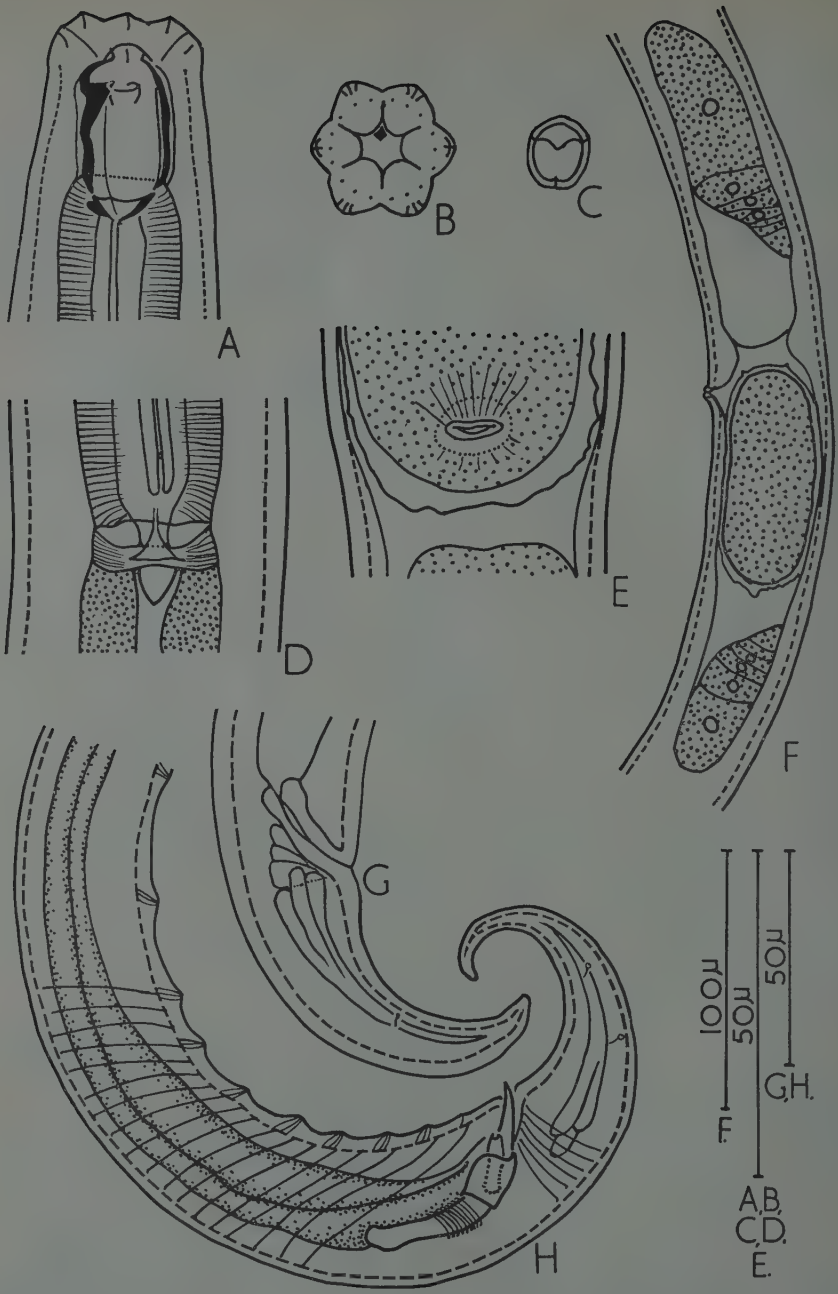

Fig. 4. *Mononchus propapillatus* n. sp. A. lateral view of head, B. *en face* view of lip region, C. stoma at level of tooth apex, D. oesophago-intestinal junction, E. ventral view of vulva, F. female reproductive structures, G. female tail, H. male tail.

Raoul forest litter; Hongi's Track, in litter of *Beilschmiedia Tawa* Benth. & Hook. f. — *Knightia excelsa* R. Br. — *Podocarpus Totara* D. Don forest; Kirkpaka in litter of *Beilschmiedia Taraire* Benth. & Hook. f. — *Vitex lucens* T. Kirk — *Corynocarpus laevigata* Forst. forest; Tutamoe, from mosses and liverworts off tree trunks in *Beilschmiedia Tawa* Benth. & Hook. f. — *Dacrydium cupressinum* Soland. forest.

Remarks. This species, like *M. composticola* is found in native forests, but is associated with the cryptogamic flora as well as with the litter zone, and is more abundant in the North Island than in the South Island.

The specific name refers to the glands about the median vulva (mes = middle, adenus = a gland).

Females collected in April 1958 had spermatozoa in the uterus and oviduct.

Mononchus propapillatus n. sp.

Figs. 4A-H.

Holotype ♀ L = 1.16 mm; a = 22; b = 3.5; c = 12.6; V ^{24} 61.5 ^{14}.
Allotype ♂ L = 1.10 mm; a = 32; b = 3.5; c = 11.5; T = 39.

For measurements of paratypes see Table III.

Cuticle smooth, without striations, 2 μ thick. Lateral fields not detected in either sex. Subcuticle 1.5 μ thick. Head offset by a moderate constriction, lips distinguishable in lateral view. Amphid opening slit-like, situated at the level of the dorsal tooth apex, and immediately posterior to the outer lateral papilla. Amphid narowing to half aperture width at a distance equal to amphid width; amphid obscure beyond this point.

In *en face* view lip region hexagonal, symmetrical; all six lips similar, rounded on both edges. Papillae in two rings; an inner ring of eighteen, three on each lip arranged in a straight line across the lip, and an outer ring of fourteen, three on each of the submedian lips and one on each of the laterals. Amphid apertures crescentic in this view. Anterior rim of sclerotized stoma conspicuous; the sutures between the three parts coincide with the clefts between the subdorsal and lateral lips, and between the subventral lips. At the level of the dorsal tooth apex the stoma is rather oval in cross section, and is slightly indented ventrally. This indentation bears the ventral ridge seen in lateral view. Near its apex the tooth spreads over the whole of the dorsal wall of the stoma. In lateral view the heavily sclerotized stoma occupies half the body width, is roughly oval in shape, but rather blunter anteriorly than posteriorly. The anterior aperture is circular and approximately one third of the greatest stoma width. The dorsal wall bears the large, single tooth whose anterior edge is straight and which slopes slightly posteriorly from the apex. Posterior edge or buttress of the tooth joins the lateral wall slightly anterior to the mid point of this wall. In the region of the tooth the outer surface of the wall is slightly indented. Ventral wall curves smoothly throughout its length, and from the posterior limit of the anterior element to a point nearly

TABLE III

Measurements of *Mononchus propapillatus* n. sp. females

	Mt. Cook	Waitaanga Saddle	Kumara Beach	Kiripaka	Waimangu	Mangatere	Kerikeri	Craigielachie	Takaka	Tikipunga	Levin Bush
n	24	13	12	8	7	6	5	5	6	3	1
L (mm) (mean)	1.15	1.57	1.21	1.02	1.09	1.27	0.89	1.19	1.01	1.27	1.03
S.D. (mm)	± 0.060	± 0.132	± 0.058	± 0.057	± 0.078	± 0.089	± 0.037	± 0.024	± 0.051	± 0.041	—
range	1.01–1.71	1.39–1.78	1.12–1.32	0.93–1.11	0.95–1.18	1.21–1.38	0.84–0.93	1.14–1.21	0.93–1.07	1.08–1.38	1.03
a	23–33	27–34	24–36	25–31	27–28	24–29	26–29	25–30	24–28	29–30	21
b	3.24–3.81	3.4–3.98	3.30–3.71	3.21–3.45	3.00–3.44	3.52–3.89	3.18–3.23	3.34–3.47	2.95–3.41	3.48–3.87	3.62
c	11.9–16.5	10.1–12.8	13.2–18.9	10.7–13.3	11.2–13.3	13.8–15.7	10.3–11.6	14.8–17.4	12.1–13.8	12.8–15.0	10.7
a.o.	8–16	7–19	10–14	8–15	7–13	8–12	10–13	10–16	9–14	10–11	12
V	57.7–62.9	58.6–69.1	61.1–62.6	61.2–64.1	59.7–63.4	59.5–65.5	60.2–61.9	61.8–64.0	57.8–63.3	61.0–61.7	61.6
p.o.	10–16	8–16	8–12	9–12	7–12	7–20	8–13	9–15	10–15	10–11	18

opposite the tooth apex is a low untoothed ridge. The posterior elements make up the base of the stoma; the dorsal element is greatly thickened anteriorly, but the ventro-lateral elements are not. All three posterior elements are continuous with the cuticular lining of the oesophagus. Anterior three-quarters of stoma surrounded by a muscle sheath; posterior quarter embedded in the fore end of the oesophagus.

Oesophagus slightly expanded anteriorly, then narrowing a little to the nerve ring at one third its length; expanding slightly beyond this in conformity with the general body contour, and occupying half the body width.

Oesophago-intestinal junction typical of the genus, as shown in figure 4 D. Intestine a simple tube; cells packed with fine granules.

Female. Vulva a transverse slit with thickened lips which make it conspicuous even in lateral view. Vagina thick-walled and extending one third of the body-width. Uteri bifid, size very variable with the stage of development of the eggs. Ovaries paired, opposed and reflexed through three-quarters of their length. Ovaries almost three quarters as wide as the body, ova in a single row within each ovary. Usually only one mature egg at once, rarely two. (Material collection in February). Intestine passes to the left of the anterior and to the right of the posterior ovary. No prerectum in female. Rectum straight.

Tail conoid, ventrally arcuate, two and a half times anal body width in length, tip rounded. Caudal glands present, terminal duct absent. Caudal cuticular pores not seen.

Males. Testes paired, outstretched in opposite directions; anterior testis five-sixths as long as posterior testis; sperms numerous, not arranged in any definite pattern. Vas deferens seven-eights of testicular length. From a point opposite the second supplement the vas deferens swells to become the ejaculatory duct which opens into the cloaca. Pre-rectum as long as ejaculatory duct.

Spicules long, arcuate, moderately slender, upper half embedded in a muscular sheath. Gubernaculum large, complex, consisting of a median dorsal portion from which arise two broad strap-like lateral processes which curve around the spicules. From the outer surface of each lateral process arises a ventrally directed accessory piece which is bifid at the tip. Supplements, nine papilliform, and one adanal without a papilla. The anterior three more widely spaced than the remainder.

Tail similar to female but tip more pointed. Caudal papillae, one dorsal pair at one-third of tail length and one lateral pair at threefifths of tail length.

Differential diagnosis. Mononchus propapillatus differs from all other species in the genus, except *M. papillatus* Bastian in the possession of an untoothed ventral ridge. It is close to *M. papillatus* from which it is distinguished on male characters; viz. the presence of lateral accessory pieces; a gubernaculum with curved lateral processes which curve around the lateral accessory pieces and the spicules, and only ten supplements, of which the adanal member is not papilliform. *M. papillatus* has no lateral accessory pieces, the gubernaculum is simple, without lateral processes, and lies wholly above the spicules; the supplements are

twelve to sixteen in number and uniform in type. The lips of *propapillatus* are rounded, and the stoma oval in cross section, but in *papillatus* the lips are angular, and the stoma squarish.

The females of *M. propapillatus* are so similar to those of *M. papillatus* that I hesitate to erect a new species, but in view of the dissimilarity of the males there is no alternative as there is no doubt that they are correctly associated with the females. New Zealand has been isolated from other land masses since Cretaceous times and, on virgin sites, it is reasonable to expect slightly different forms. There is no reason why specific differentiation should not go further in one sex than in the other. There is no evidence of male dimorphism as found in the Acari (ANDRÉ, 1949) and the Opiliones (FORSTER, 1954) as only one type of male is known for each species.

Type slides. Holotype, allotype and paratypes are in the nematode collection, Entomology Division. Paratypes also at Nematology Department, Rothamsted Experimental Station; Slide nos. 143/6/1-1.

Type locality. Governor's Bush, Mount Cook, in *Nothofagus Menziesii* Oerst. forest with *Podocarpus Hallii* T. Kirk., *Phyllocladus alpinus* Hook. f. and *Coprosma* spp. Forst. from surface three inches of litter and soil. Soil type: Haast fine sandy loam (a semi-mature podzol derived from greywacke and schist). Height 810 metres.

Other localities. (a) South Island: Takaka Hill in *Nothofagus fusca* Oerst. forest; Whangamoa Hill in reverting grassland; Kumara Beach, *Trifolium repens* L. — *Hydrocotyle* sp. L. dominant grassland; Charleston Beach in *Lotus uliginosus* Schkuhr.; Ross in *Anthoxanthum odoratum* L. — *Trifolium repens* L. pasture; Cape Foulwind in *Agrostis stolonifera* L. — *Holcus lanatus* L. pasture; Utopia Road, Westport in *Anthoxanthum odoratum* L. — *Cynosurus cristatus* L. pasture; Granity in *Agrostis stolonifera* L. — *H. lanatus* L. pasture; Pelorus Bridge in *Podocarpus spicatus* R. Br. — *Beilschmiedia Tawa* Benth. & Hook. f. forest; Craigielachie, *Dactylis glomerata* L. — *Agrostis tenuis* Sibth. pasture; Cass in *Agrostis tenuis* Sibth. — *Anthoxanthum odoratum* L. grassland; Waikari — Culverden in *Festuca novaezealandiae* Cockayne — *Agropyrum scabrum* Beauv. grassland. (b) North Island: Levin Bush *Knightia excelsa* R. Br. — *Beilschmiedia Tawa* Benth. & Hook. f. forest; Waverly in *Lolium perenne* L. — *Trifolium repens* L. pasture; Waitaanga Saddle *Weinmannia racemosa* Linn. f. — *Brachyglottis repanda* Forst. — tree fern forest; Mangatere, *Paspalum dilatatum* Poir dominant pasture; Wharekohe, 2 year old *Lolium perenne* L. — *Trifolium repens* pasture (previously heathland type vegetation); Kiripaka in *Beilschmiedia Taraire* Benth. & Hook. f. — *Vitex lucens* T. Kirk forest; Tikipunga in *Paspalum dilatatum* Poir — *Lotus hispidus* Desf. — *Trifolium repens* L. pasture; Kerikeri in Low *Leptospermum scoparium* Forst. — *Hakea acicularis* R. Br. — *Cassinia fulvida* Hook. f. heath; Waimangu under *Pteridium esculentum* Hook. f., *Rubus* sp. L. (blackberry), *Festuca arundinacea* Schreb.

Remarks. This species is very widespread in New Zealand and its distribution

seems little influenced by factors such as soil type, vegetation cover, or climate. The specimens from Kerikeri, however, where the soil is particularly barren, are smaller than those from other localities. *Iotonchus parazschokkei* (Allgén) is also small at this site.

Prionchulus muscorum (Dujardin, 1845) Chitw. & Chitw., 1937

For synonymy see CLARK, 1960b.

Arthurs Pass 1 ♀ L = 2.8 mm; a = 34; b = 4.25; c = 9.27; V ^{11} 62.5 ^{12} stoma 47 μ.

Mt. Cook 1 ♀ L = 1.715 mm; a = 32.73; b = 3.81; c = 16.52; V ^{12} 62.1 ^{13} stoma 37 μ.

Waimungu 1 ♀ L = 1.95 mm; a = 30; b = 4.19; c = 15.9; V ^{11} 65.1 ^{12} stoma 38 μ.

Wharekohe 1 ♀ L = 1.68 mm; a = 35; b = 4.02; c = 17.3; V ^{9} 66.3 ^{7} stoma 36 μ.

Remarks. The measurements given above lie for the most part within the limits of variation given for this species by CLARK (1960b) for British specimens. The most important divergence is that for tail length in the Arthurs Pass specimen where c = 9.27 which indicates a much longer tail than is usual in European specimens. I hesitate to separate this form on the basis of a single specimen. Otherwise it is indistinguishable from European specimens. In the specimens from the other localities in New Zealand the denticulations of the ventral ridges are less well developed than in British specimens.

Other locality. Nelson, Entomology Division compost heap, larvae only.

ZUSAMMENFASSUNG

Neu-Seeländische Mononchiden (Enoplida, Nematoda) I. Die Gattungen Mononchus *Bastian und* Prionchulus *Cobb*

Mononchus composticola, M. mesadenus, und *M. propapillatus* n. spp. werden beschrieben, *Prionchulus muscorum* (Dujardin) wird aus Neu-Seeland erwähnt. *M. campelli* Allgén 1929 ist als species inquirenda anzusehen. *M. composticola* und *M. mesadenus* werden mit *M. gerlachei* de Man 1904 und *M. major* Cobb 1893 vereinigt. Durch den angegebenen Schlüssel können sie voneinander getrennt werden.

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CORRIGENDA

Nematologica **5** (1960)

p. 100. Scale attached to Fig. 1A for 0.01 mm read 0.025 mm.

p. 116. *L. moro* Fuchs, 1937
L. ulmi Kahn, 1960
are recognised as separate species not synonyms of *L. lignophilus.*

p. 120. At the end of section on *Peraphelenchus* add: No other species.

p. 131. Second line of paragraph 2 for — + — shaped see + shaped.

p. 134. Table III
line 4 to read L_4 pre ♀
line 5 „ „ L_4 pre ♂
line 6 „ „ Adults.

Nematologica 5 (1960): 215-216. E. J. Brill, Leiden

A NOTE ON PARTHENOGENESIS AND SEX DETERMINATION IN *HETERODERA ROSTOCHIENSIS* WOLL.

BY

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Investigations on the existence of parthenogenesis in *H. rostochiensis* have so far been conducted with few inoculations of single larvae only. FASSULIOTIS (1957) inoculated twenty eight plants and obtained ten females, all without eggs or larvae. ELLENBY (1957) inoculated forty five plants with single larvae three of which formed cysts also devoid of eggs or larvae, WILLIAMS (1957) found two females without eggs after inoculation of sterile half tubers with single larvae. Although the results of these experiments show that as a rule males are necessary for the multiplication of *H. rostochiensis*, they are not sufficient to support the view that parthenogenesis is rare or does not occur.

About 700 potato seedlings were planted in small (6 x 18 cm), aerated agar plates in plastic bags (DEN OUDEN, 1960). Two weeks after planting, these seedlings which had then developed roots several centimetres long, were inoculated with one, freshly hatched larva of *H. rostochiensis* each. These larvae were placed on the agar close to a root tip.

Four weeks after the inoculation 686 plants had survived. On the roots of 270 of these a female was found very close to the place of inoculation. In only one of the other bags was a male observed. It could not be decided whether there had been more males originally, which had wandered away in the agar or whether practically all larvae which had succeeded in entering a root had become females. It is improbable, however, that practically all of more than 200 males would have escaped attention and it must be assumed that most larvae which invaded the roots became females. As many males occur, when a plant is inoculated with a great number of larvae, this suggests that the likelihood of a larva becoming male or female depends on external conditions, presumably abundance of food. Such a phenomenon has been reported before.

COBB, STEINER & CHRISTIE (1927) infected the grasshopper *Melanoplus femur-rubrum* Deg. with eggs of the parasitic nematode *Mermis subnigrescens* Duj. When twenty eggs were fed to a grasshopper free of this parasite, the result was nineteen parasites, all males. Feeding a very few eggs resulted in female parasites. Similar observations were made on other mermithids, infecting bugs and ants. According to ELLENBY (1954) the percentage of males in a population of *H. rostochiensis* increases with increasing density of this species in the roots. TRIANTA-

PHYLLOU & HIRSCHMANN (1959) found that the ratio of males to females in *Meloidogyne incognita* and *M. javanica* is increased by unfavourable conditions.

The plants were kept growing for another 6 weeks after the first inspection and then left to dry out slowly. Three months after inoculation not all the females had turned brown. ELLENBY (1957) and WILLIAMS (1957) found the same. Of the 270 females observed four weeks after inoculation, 246 were found again two months later; 39 % of these were of normal size and shape, 37 % of medium size and 17 % small but of normal shape; 7 % were small and ellipsoid. None of them contained eggs. When opened in water a granular mass came out in which ovaria and oöcytes could still be observed. One may therefore conclude that in the population of *H. rostochiensis* investigated here parthenogenesis is either very rare or does not occur at all.

ZUSAMMENFASSUNG

Über Parthenogenese und Geschlechtsbestimmung bei Heterodera rostochiensis *Woll.*

An 270 von 700 mit einer Larve von *Heterodera rostochiensis* infizierten Kartoffelpflanzen, die auf Agarplatten gezüchtet wurden, wurde nach vier Wochen ein Weibchen gefunden, und nur bei einer Pflanze ein Männchen. Auf Agarplatten infiziert mit vielen Larven wurde immer eine grosse Zahl Männchen gefunden. Offenbar wird die Entwicklung von Larve zu Männchen oder Weibchen durch Umweltbedingungen bestimmt.

Von den 270 Weibchen wurden drei Monate nach der Inokulation 246 wiedergefunden; 39% waren von normaler Grösse, 37% von mittlerer Grösse und 17% klein. 7% waren nicht völlig entwickelt. Keine der 246 Zysten enthielt Eier oder Larven; Parthenogenese kommt also bei *H. rostochiensis* nicht oder nur vereinzelt vor.

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SHORT COMMUNICATION

ALAN F. BIRD [1]), *Additional notes on the attractiveness of roots to plant parasitic nematodes.*

KLINGLER (1959) reported that the stem nematode *Ditylenchus dipsaci* is attracted to carbon dioxide introduced through a fine capillary into soil. BIRD (1959), however did not find carbon dioxide in bicarbonate buffered, 1 % agar discs consistently attractive to *Meloidogyne javanica* larvae. Because Klingler's method allows a continuous gradient to be maintained, it was used in further tests on the response of larvae of *M. javanica* to carbon dioxide.

Carbon dioxide was introduced through a fine capillary into 0.25 % agar containing the test specimens. Pressure was adjusted so that small quantities of carbon dioxide diffused through the agar in the tube and into the agar in the petri dish containing the specimens. Using this technique carbon dioxide was found to attract the larvae of *M. javanica* which grouped around the tip of the capillary tube; it also attracted various other species of nematodes such as the larvae of *Heterodera schachtii,* larvae and adults of *Pratylenchus minyus,* pre-adult larvae of *Paratylenchus* sp. and various rhabditids.

Carbon dioxide may play a part as a general, long range attractant to many nematodes under the conditions prevailing around plant roots. However, localization of nematodes in the region of cell elongation does not appear to be influenced by carbon dioxide as the redox potential is lowest here. If carbon dioxide were present in greater concentrations in this region of the root, it would tend to raise the redox potential. Also, a piece of living root tip attracts *M. javanica* larvae away from the tip of a capillary through which carbon dioxide is diffusing.

As only *M. javanica* and *H. schachtii* of the above mentioned nematodes are attracted to agar discs of 0.1 M sodium dithionite it would seem that lowered redox potential is important in the attraction of these two species. However, not all reducing agents attract these nematodes, and of those that do, not all do so on a scale equivalent to their reducing abilities (BIRD, 1959). Attractants such as sodium dithionite are not physiologically active and it seems that root exudations must play a more important part than previously suspected (BIRD, 1959), whilst lowered redox potential, which appears to influence members of the family Heteroderidae, may not be as important as previous experiments indicated.

Places in plant roots that are most attractive to larvae of *M. javanica* are the region of cell elongation and the apical meristem in front of it, points where lateral roots are emerging, and freshly cut root surfaces. These places may owe their attractiveness to the exudation of attractant substances which may be reducing agents and which seem to be associated with or close to areas of meristematic activity.

It seems that attraction of plant parasitic nematodes to roots is due to responses to different combinations of factors which vary according to the species. These factors may include carbon dioxide, lowered redox potentials and substances such as glutamic acid (BIRD, 1959) and its derivatives as well as other substances associated with root metabolism. In the case of those nematodes attracted to the region of cell elongation and the active meristematic region which precedes it, precise knowledge of these attractants depends on their identification and determination of the concentrations to which nematodes are sensitive.

I thank Mr. P. P. Thomson for technical assistance and Mr. J. Fisher of the Waite Agricultural Research Institute for providing specimens of *Pratylenchus minyus* and *Paratylenchus* sp.

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ANNOUNCEMENT FROM THE EUROPEAN SOCIETY OF NEMATOLOGISTS

Secretary: Ing. K. Lindhardt, Plant Pathology Institute, Lyngby, Denmark.

Sixth International Nematology Symposium, Ghent (Belgium), 25-28 July 1961.

PRELIMINARY PROGRAMME

Monday, July 24: Arrival of participants

Tuesday, July 25: Morning — Inaugural address
Afternoon — Lectures (Root-knot nematodes)

Wednesday, July 26: Morning — Lectures (Cyst-forming nematodes)
Afternoon — Lectures (Techniques)
Visit to the Laboratories of the State Agricultural University

Thursday, July 27: Morning — Lectures (free-living nematodes)
Afternoon — Lectures (Systematics, Ecology, etc.)
Visit to the State Plant Breeding Station (R.V.P.) Lemberge.

Friday, July 28: — Whole day excursion: Visit to field trials. Ghent — Belgian Coast — Bruges — Ghent.

The Symposium fee will be 200 Belgian Francs (members) or 240 B.F. (non-members).

Titles to lectures, accompanied by summaries in French, English and German languages, are to be sent before April 1, 1961; applications to be sent before June 15, 1961 to the Secretary of the Symposium, c/o Rijkslandbouwhogeschool, Coupure Links 233, Ghent, Belgium.

Prof. J. van den Brande
Chairman

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE

Notice of proposed use of plenary powers in certain cases (*A.* (*n.s.*) *45*)

In accordance with a decision of the 13th International Congress of Zoology, 1948, public notice is hereby given of the possible use by the International Commission on Zoological Nomenclature of its plenary powers in connection with the following cases, full details of which will be found in Bulletin of Zoological Nomenclature, Vol. 17, Parts 9/11 to be published on 16th September 1960.

Designation of a type-species for the nominal genus *Blankaartia* Oudemans, 1911 (Nematoda) Z.N.(S.) 330

Anyone who wishes to comment on the above should write in duplicate as soon as possible giving the reference number of the case in question. Comments received early enough will be published in the *Bulletin of Zoological Nomenclature*. Those received too late for publication will, if received before 16th December 1960, be brought to the attention of the Commission at the time of commencement of voting.

The Secretary,
International Commission on Zoological Nomenclature
c/o British Museum (Natural History),
Cromwell Road,
London, S.W. 7,
England.

Nematologica 5 (1960): 219-227. Leiden, E. J. Brill

INHIBITION OF ROOT-KNOT DEVELOPMENT ON TOMATO BY SYSTEMIC COMPOUNDS

BY

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PEACOCK (1959) described a technique for culturing the root-knot nematode *Meloidogyne incognita* (Kofoid and White) under controlled conditions. One object of this technique was to study the effect on parasite development and host plant reaction (i.e. gall formation) of test substances introduced into the nutrient medium in which excised host (tomato) roots were cultured. Experiments were made to test theories of possible mechanisms of interference with the normal nematode/host root complex. Test substances were also introduced into intact tomato plants via a wool "wick", and finally, some were applied to the leaves.

Plant growth regulators were first tested because evidence exists for their participation in insect-induced plant galls (NYSTERAKIS, 1948; BOYSEN-JENSEN, 1948; CAMBAR, 1953). WARTENBERG (1954) thought galling and tissue abnormalities produced by *Eriosoma* were caused by inhibition and modification of the normal plant hormone system and not by the injection of phytohormones by the insect, but LEATHERDALE (1955) demonstrated the presence of a growth-regulating substance in a cell-free extract of fully-grown Cecidomyid larvae and its ability to promote plant cell-division. NOLTE (1952) reported that plants infected with *Ditylenchus dipsaci* have a higher content of hetero-auxin or similar growth substance than have uninfected controls. Presumably this auxin could be injected by the nematode, or accumulate by the nematode blocking the vascular system of the plant. Conversely, certain aspects of the morpho-pathology of nematode galls, in particular the formation of side-roots from knots caused by *Meloidogyne* spp. might be explained by postulating an auxin antagonist in nematode "saliva".

Some glycosides were tested because of their common occurrence and possible downward translocation in plants and because on hydrolysis (which might be induced by parasite damage to the root) they could yield compounds toxic to nematodes, e.g. isothiocyanates from sinigrin, prussic acid from amygdalin, sambunigrin. ROHDE & JENKINS (1958) extracted from roots of *Asparagus officinalis* (resistant to the stubby root nematode, *Trichodorus christiei*) a glycoside toxic to several plant parasitic nematodes. Chloral hydrate initiates the formation of several glycosides in plants (MILLER 1943, 1947), and was therefore included in these experiments.

Malachite green and diethyl malonate were included as inhibitors of respiratory

enzymes, as temporary disorganisation of these processes might produce metabolites inimical to the parasite. Streptomycin sulphate induces resistance to blight (*Phytophthora infestans*) in tomato and potato which resistance is correlated with higher polyphenolase activity (Voros, Kiraly & Farkas 1957). Caffeine and xantheine upset the ribonucleic acid/deoxyribonucleic acid ratio in treated apple leaves and thereby reduce aphis populations on the leaves (Kessler, Swirski & Tahori, 1958). In the present work hypoxantheine was substituted for xantheine which was too sparingly soluble. Thiouracil inhibits the development of cucumber mosaic virus in tobacco by blocking protein synthesis (Porter & Weinstein 1957); 6-methoxy benzoxazolinone is a naturally occurring stem-borer-resistance factor isolated from maize (Smissman, Lapidus & Beck 1957) and a related compound, 2 (3) benzoxazolinone is responsible for resistance to *Fusarium* sp. in rye seedlings (Virtanen & Hietala 1955).

In addition to tests with these potential chemical inhibitors, attempts were made to upset the nutrition of the parasite by reducing the vitamin content of the host, by grossly increasing the amino-acid content (of the medium) and by supplying carbohydrate of a different type. Extracts and leachates of nematode-resistant plants were tested, and a small number of compounds was also tested for ability to break the resistance to root-knot eelworm of *Lycopersicon peruvianum* roots in culture medium.

METHODS

Root cultures

Excised tomato root tips were grown in White's nutrient medium to which test chemicals had been added; after 8 days they were exposed to *Meloidogyne incognita* larvae for 24 hours and then returned to unadulterated White's medium (cf. Peacock 1959). Observations were made after exposure and again at intervals over the next 4-6 weeks. The object of the treatment was to inhibit attraction of the larvae to the root tip, penetration, swelling, and development of the complex structure of the "gall".

Experience with this technique showed that a number of aberrant host/parasite relationships could and did develop. Apparently identical root tips placed on thin agar either (i) grew normally, (ii) replaced apical growth by growth of side-shoots, (iii) ceased to grow. Also, larvae entering the root gave rise to either a spherical swelling from which the root tip grew away, and whose development could be studied, or a terminal diffuse swelling caused by simultaneous invasion of large numbers of larvae, which prevented further elongation of the root and the development of which varied considerably. In consequence, the interpretation of results was not always easy and many tests had to be repeated.

Wick Feeding

Tests were made with various techniques to encourage the translocation of test compounds to the roots of intact plants, and it was found that certain dyes, notably

acid and basic fuchsin and aniline blue, when fed to the base of tomato stem through a wool "wick" at 1,000 ppm. appeared at the root tips after 48 hours. At 100 ppm. they were observed in the stem base below soil level and in the upper roots. The same dyes failed to move to tomato roots when leaves, intact or cut, or the whole plant tops, were immersed for 24 hours in the dye having previously been washed for five minutes in 0.1 % Tween 20, a wetting agent. After 7 days, dyes which had reached root-tips in the first 48 hours were still visible; those which had not, were still not detectable. Wick feeding through, above, or below the first stem node gave similar results.

Test compounds were then wick-fed to tomato plants at 1,000 and 100 ppm. or applied as a soil drench to give 25 ppm. in the soil, a rate at which only a potent nematicide might be expected to be effective. The soil around the plant was inoculated with 24-hour-old *Meloidogyne* larvae (500-600 per plant) 48 hours after the chemical treatment was begun and the plants were examined 12 days after inoculation.

Foliar application

Nusbaum (1958) reported that maleic hydrazide applied to the leaves of tobacco inhibited development of *Meloidogyne* sp. in the roots. Baranyovits (private communication) has also shown inhibition of development of *H. rostochiensis* on tomato roots following foliar and other applications of a number of compounds, including maleic hydrazide and diethyl malonate. Maleic hydrazide was now examined as a foliar spray (4.0 % acetone/0.01 % Agral emulsifier in water) and as a lanoline smear on the leaves, compared with a wick-fed application. Spray was delivered from a paint spray gun at 25 lb. sq. in. and leaves were sprayed to "run-off", the soil being protected from spray. The experiment also compared the effect of application 2 days before inoculation of the soil with root-knot larvae and 2 days after inoculation. 500-600 24-hour-old larvae per plant constituted the inoculum. Following this experiment, the other active chemicals were then examined as foliar sprays. Roots were washed and galls counted 12-15 days after inoculation.

results (Table I)

In the excised root test, so long as root growth was maintained, normal host-parasite relationship developed whatever the treatment, except for parathion, a powerful nematicide which may have been present in toxic quantity on the surface of the root. Attempts to check the parasite by changing the amino-acid content of the host or to introduce a resistance factor from extracts and leachates of nematode-resistant plants (*L. peruvianum, Tagetes* sp., *Pelargonium* sp.) were similarly unsuccessful. So also were attempts to break the resistance of excised roots of *L. peruvianum* by growing them in nutrient solution plus anyone of the following: ascorbic acid, tryptophane, arginine, casein hydrolysate, yeast extract, digitalin and amygdalin.

When test chemicals were wick-fed to the stems of intact tomato plants, maleic hydrazide inhibited root-knot eelworm development completely at 1,000 ppm.

TABLE I

Inhibition of root-knot development on Tomato by systemic compounds

Treatment	*Treatment added to culture medium* Growth rate diminished at ... ppm.	Abnormal growth if any	Attraction, invasion — development of galls	*Treatment wick-fed to intact plant* A (1000 ppm.) B (100 ppm.) and applied to soil C (25 ppm.) *Root grading* [1]) Application: A	B	C	*Root-knot grading* [2]) Application: A	B	C
Control	—	—	Normal	5	5	5	1	1	1
Indoleacetic acid	0.125	apical swelling	Normal to 0.125 ppm.	1	5	5	—	1	1
Methoxyphenylacetic acid	5.000	apical dominance diminished	Normal to 10.000 ppm.	4	5	3	1	1	1
Naphthalene acetic acid	0.005	—	Normal to 0.005 ppm.	1	4	3	—	1	4
1-Naphthoxy acetic acid	0.500	apical swelling	Normal to 0.500 ppm.	4	5	3	1	1	1
2 : 3 : 6-Trichlorophenyl acetic acid			not tested	3	3	3	3	1	1
2 : 4-Dichlorophenoxyisobutyric acid	2.000	—	Normal to 2.000 ppm.	2	5	5	—	1	1
2-4-Dichloroanisole			not tested	1	5	5	—	3	3
2-4-6-Trichloroanisole			not tested	5	5	5	1	1	1
2-3-5-Tri-iodobenzoic acid			not tested	1	2	5	—	1	1
Gibberellic acid	—	—	Normal to top rate 10 ppm.	5	5	5	1	1	1
α(1-naphthylmethyl sulphide) propionic acid	4.000	—	Normal to 2 ppm. Galls smaller at 4 ppm.	2	5	1	—	1	—
Diphenylene glycollic acid	2.500	suppressed lateral growth	Normal up to 10 ppm.			not tested			
N(1-naphthyl) phthalamic acid	1.25	suppressed lateral growth	Normal up to 2.5 ppm.			not tested			
Quinic acid	—	—	Normal to top rate 10 ppm.			not tested			
Maleic hydrazide	5.00	apical swelling apical dominance diminished	Normal to 5 ppm.	3	3	5	5	4	1
6-Furfurylaminopurine (Kinetin)	1.25	sub-apical swelling	Normal at 1.25 ppm.	1	1	4	—	—	3
Diethyl malonate	10.00	suppressed lateral growth	Galls smaller at 10 ppm.	5	5	5	1	1	1

Caffeine	100.00	—	Normal to 100 ppm.	5	4	4	2	1	1
Hypoxantheine	—	—	Normal to 100 ppm.	5	5	5	1	1	1
Coumarin	5.00	—	Normal to 5 ppm.						
			Galls smaller at 10 ppm.	3	5	5	5	1	1
Thiouracil	1.56	—	no results	1	5	2	—	1	5
Streptomycine sulphate	12.50	—	Normal to 12.5 ppm.	5	5	5	1	1	1
2 : 4 : 5-Trichlorobenzene sulphonamide			not tested	5	5	5	1	1	1
2 : 4 : 6-Trichlorobenzene sulphonamide			not tested	2	4	5	—	1	1
Chloral hydrate	50.00	—	Normal to 50 ppm.			not tested			
Methylamine	2.50	—	Normal to 5 ppm.			not tested			
Malachite green	0.10	—	Normal to 0.4 ppm.			not tested			
Benzoxazolone	100.00	—	Normal to 100 ppm.	5	5	5	1	1	1
2-Methyl benzoxazole	100.00	—	Normal to 100 ppm.	5	5	5	1	1	1
Parathion	—	—	at 20 ppm. (sat^{n}) few						
			galls and these small	5	5	5	3	1	1
Sodium fluoroacetate	10.00	—	Normal to 10 ppm.	5	5	5	5	5	4
Fluoracetamide	40.00	—	Normal to 40 ppm.	5	5	5	5	3	4
Digitoxin	5.00	—	Normal to 5 ppm.	5	5	5	1	1	1
Digitalin	5.00	—	Normal to 5 ppm.	5	5	5	1	1	1
Sinigrin	—	—	Normal to top rate 10 ppm.	5	5	5	1	1	2
Amygdalin	—	—	Normal to top rate 50 ppm.	5	5	5	1	1	1
Salicin	5.00	—	Normal to 5 ppm.	5	5	5	2	1	1
Convallotoxin	—	—	Normal to top rate 20 ppm.	5	5	5	1	1	1
Strophantin-G	—	—	Normal to top rate 10 ppm.	5	5	5	1	1	1
Mannitol	—	—	Normal to top rate 200 ppm.	5	5	4	1	1	3

[1]) *Root grading:* 1. Plant dead; 2. Plant alive, but root growth negligible; 3. Root growth poor to moderate; 4. Good root, though phytotoxic symptoms; 5. As good, or better than control.

[2]) *Root-knot grading:* 1. Galls as numerous, or more so than control; 2. Som reduction; 3. Reduced to 25-50 % of control; 4. Few galls for size of root 5. No galls.

TABLE II

Effect on development of root-knot on tomato of maleic hydrazide foliage treatments. Observations 15 days after inoculation. 4 replicates.

				Plants inoculated with root-knot larvae 2 days *before* treatment			Plants inoculated with root-knot larvae 2 days *after* treatment		
Treatment	*Application*	*Dilution* ppm.	mg. act. ingred. per plant	*Root grading* [1]	*Foliage grading* [2]	*No. of galls per plant*	*Root grading* [1]	*Foliage grading* [2]	*No. of galls per plant*
Control	—	—	—	5	5	125.0	5	5	93.5
Maleic Hydrazide	Wick-fed to stem	100	0.45	3	5	123.5	4	5	41.0
		1000	4.50	2	5	7.0	3	5	0.0
	Sprayed on leaves	1000	2.50	4	5	79.5	4	5	14.0
		10,000	25.00	3	4	9.0	3	4	0.0
	To leaves in lanoline	56,000	14.50	4	4	104.0	5	4	71.5

[1]) *Root grading*
1. Plant dead
2. Plant alive but root growth negligible
3. Root growth poor to moderate
4. Good root, though phytotoxic symptoms.
5. As good or better than control.

[2]) *Foliage grading*
1. Plant dead
2. Severe scorch, growing point dead
3. Moderate scorch
4. Tip scorch only
5. As control

and almost completely at 100 ppm., but this effect was accompanied by marked inhibition of root growth. Coumarin also completely inhibited root-knot development at 1,000 ppm. wick-fed, whilst causing severe phytotoxicity; no effect was detectable at 100 ppm. 2 : 4-dichloroanisole slightly inhibited root-knot development at 100 ppm. but killed the plant at 1,000 ppm.: it was also nematicidal in soil at 25 ppm. Parathion inhibited root-knot development to a certain extent when wick-fed at 1,000 ppm. but not at 100 ppm. Sodium fluoracetate completely inhibited root-knot development at 1000 and 100 ppm. without phytotoxicity, and fluoracetamide was almost as effective. Both of these compounds were nematicidal in soil at 25 ppm.

Foliar application of maleic hydrazide was more effective as an aqueous spray than as a lanoline smear (Table II). To achieve a similar effect to that of wick-feeding, a foliar spray required, in terms of mg. active ingredient per plant, some five times as much material, but since so much less material is retained by the sprayed plant the concentration of active ingredient in the spray must be increased ten-fold. At this concentration some foliage scorch was evident (see also Table III

TABLE III

Effect on development of root-knot on tomato of chemicals sprayed on the leaves. Observations 14 days after inoculation.

Treatment	*Dilution* ppm. act. ingred.	*Plant grading* *Root* [1]	*Foliage* [1]	*No. of galls per plant*
Sprayed on leaves to drip-point 2 days before inoculation of soil with root-knot larvae. Soil protected from spray.				
Control	—	5	5	105.0
	1,000	4	5	35.5
Maleic hydrazide	5,000	4	4	4.5
	10,000	4	2	4.0
	1,000	5	4	55.0
Sodium fluoroacetate	5,000	5	3	1.0
	10,000	5	3	0.5
	1,000	5	5	121.0
Fluoracetamide	5,000	5	4	59.0
	10,000	5	4	13.0
Sprayed on leaves to drip-point 4 days before inoculation of soil with root-knot larvae. Soil protected from spray.				
Control	—	5	5	147.0
	2,500	5	5	as control
2 : 4-Dichloroanisole	5,000	5	4	98.0
	10,000	4	4	36.0
	2,500	5	5	as control
Coumarin	5,000	4	4	as control
	10,000	4	4	as control

[1]) See footnotes to Table II.

where this was more marked). All applications 2 days before inoculation of the soil with *Meloidogyne* larvae were more effective than applications 2 days after inoculation. Table III summarises results of experiments with a number of chemicals applied as foliar sprays before inoculation of the soil with *Meloidogyne* larvae. Sodium fluoroacetate was about as effective as maleic hydrazide — 5000 ppm. giving almost complete protection — and less phytotoxic. Fluoracetamide and 2 : 4-dichloroanisole at 10,000 ppm. gave appreciable protection but coumarin was ineffective. At effective dosages the chemicals were all phytotoxic to some extent.

DISCUSSION

The failure of the excised-root technique to indicate compounds which, in intact plants, moved to the roots and inhibited root-knot development shows a marked difference in effect between active downward translocation in the intact plant as compared with generalised diffusion in the excised root piece. Support for the suggestion that downward movement has occurred in intact plants by translocation linked to photosynthesis is given by repeated observation that the systemic inhibitory effect of a given compound may disappear under winter conditions of low light intensity, and that this may be at least partly prevented by supplementary artificial lighting.

Maleic hydrazide has low 'in vitro' toxicity to *Meloidogyne* larvae, some 5 % mortality at 1000 ppm. maleic hydrazide having been recorded. Thus the reduction in galling on plants sprayed with maleic hydrazide is unlikely to be due to a systemic nematicidal effect unless a toxic metabolite is formed in the plant. That this also is unlikely is suggested by the difference in effectiveness of maleic hydrazide sprays before and after inoculation with eelworm larvae. Either the attractiveness of the root to larvae or its ability to respond to the presence of the parasite, is reduced by the treatment.

Sodium fluoracetate and fluoroacetamide on the other hand, are highly nematicidal 'in vitro' (roughly equivalent to mercuric chloride), and their mode of action may be more straightforward.

It is unlikely that any of these chemicals could be used practically for nematode control — they are either too phytotoxic or too hazardous. They do, however, illustrate the feasibility of a downward-moving systemic nematicide.

I thank Dr. F. Baranyovits for permission to refer to his unpublished work, and Miss Jean Taylor and Mr. J. Clayton for assistance.

RÉSUMÉ

Inhibition de la formation de galles sur les racines de tomates par des produits systémiques

Des bouts de racines de tomates croissant dans le milieu nutritif de White, furent inoculés par des larves de *Meloidogyne incognita* (Kofoid & White). L'addition de produits chimiques à cette solution ne dérangea pas les rapports normaux d'hôte/parasite, tant que la croissance des racines fut maintenue.

Toutefois, l'application de ces produits chimiques sur les tiges de tomate par des mèches en laine ou sur les feuilles empêcha soit partiellement (coumarine et 2 : 4 dichloro-anisole) soit totalement (hydrazide maleïque, fluoracetamide, fluoracetate de sodium) le développement des galles radiculaires. Au dosages effectifs tous ces produits chimiques se révélèrent phytotoxiques dans une certain mesure.

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HISTOPATHOLOGY OF GARDENIA (*GARDENIA JASMINOIDES VEITCHI*), INFECTED WITH THREE SPECIES OF *MELOIDOGYNE* [1])

BY

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The root-knot disease, caused by *Meloidogyne* spp. was first reported on gardenia by BEIJERINCK (1887) and later by NEAL (1889) and USTINOV (1939). Many small galls were observed on the root systems, but these were not studied.

Root-knot has been investigated by a number of workers on many crops, but histopathological studies have been made on a relatively few plants, including soybean (CRITTENDEN, 1958), tomato (CHRISTIE, 1936), sweet potato (KRUSBERG & NIELSEN, 1958), oat (FERVER & CRITTENDEN, 1958), rose (DAVIS & JENKINS, 1960 and REYNOLDS, 1950), and *Nicotiana* hybrids (KOSTOFF & KENDALL, 1930).

The present work was done to determine whether gall formation in gardenia was similar to that in other crops, and to determine what differences, if any, existed between galls caused by different species of *Meloidogyne.*

MATERIALS AND METHODS

Gardenia cuttings (*Gardenia jasminoides veitchi,* fam. Rubiaceae), rooted in a steam-sterilized sand, were placed into clay pots half-full of steamed soil. Each rooted cutting was inoculated by pouring directly over the roots a water suspension of roots and root-knot nematodes comminuted in a Waring blendor. Inocula were obtained from tomato plants individually infected with three species of root-knot nematode and maintained in the greenhouse. The species used were *M. hapla* Chitwood, *M. incognita incognita* (Kofoid & White), and *M. incognita acrita,* Chitwood. Pots were then filled with soil and placed in a greenhouse maintained at 21°-24°C (70°-75°F.) night temperature. After two months, galls were picked off and they and portions of check roots fixed in RANDOLPH's Modified Navashin Fluid (1935) for 24 hours. The plant material was then dehydrated by a tertiary butyl series, imbedded in Fisher Tissuemat, and sectioned at 10 μ. Sections were stained with JOHANSEN's quadruple stain (1940) and mounted in permount.

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[1]) Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers-The State University.

Figure 1. Photomicrographs of cross sections of gardenia roots infected with root-knot nematodes. A & B, *Meloidogyne incognita acrita*: C & D, *M. incognita incognita*. A. Abnormal xylem surrounding a giant cell; B. Narrow, elongate parenchyma lying between three giant cells; C. Irregular thickenings of portions of parenchyma cell walls (arrow) located within the stele and in proximity to two imbedded females; D. A group of parenchyma cells enclosed by a thickened wall (arrow). Cell walls of several enclosed cells disintegrated while remaining cell walls thickened as is illustrated in Fig. 2, E.

Figure 2. Photomicrographs of cross sections of gardenia roots infected with root-knot nematodes. E and F, *M incognita incognita*. E, Serial section approximately 100 μ from section shown in Fig. 1, D. Thickened walls of remaining enclosed cells is evident; F, Two imbedded females and three giant cells. A periderm-like development has formed on the infected side of the root.

Figure 3. Photomicrograph of a cross section of uninfected gardenia root.

RESULTS AND DISCUSSION

No differences were observed between roots infected with *M. incognita* and *M. incognita acrita.* Histological studies revealed that nematodes were generally located entirely within the stele, although occasionally their bodies extended into the cortex, particularly in young roots. Giant cells numbered from two to five to ten to twelve around a single female. They varied considerably in size with many not in contact with the head of the parasite. Young giant cells were highly vacuolated and contained thirty to forty nuclei which were larger than those in unaffected cells. In older giant cells, the number of nuclei was less, and it appeared that nuclei were coalescing. Nuclei of parenchyma cells surrounding giant cells were also much larger than normal (Fig. 3).

Cortical and stelar proliferation and hypertrophy accompanied all infections. Abnormal xylem (Fig. 1, A), formed from parenchymatous cells, was located around many giant cells and swollen females. Other stelar parenchyma cells became narrow and elongate (Fig. 1, B), their cytoplasmic contents stained deeply, and were arranged in arch-like fashion in the stele around some giant cells and nematodes. In some infected roots, alongside or in close proximity to giant cells, there were irregular thickenings of portions of cell walls (Fig. 1, C), which sometimes enclosed groups of parenchyma cells (Fig. 1, D). Enclosing of groups of parenchyma cells by thickened areas has been reported by Davis & Jenkins (1960). The number of enclosed cells decreased, due to breakdown of some cell walls as they proceeded from the head of the parasite, until only a few cells remained (Fig. 2, E). Very often, cell walls of the remaining enclosed cells thickened considerably. Giant cells were not observed to have formed from these areas.

The disruption of the stele by giant cells, proliferated parenchyma cells, and the parasites themselves caused the conducting tissues to be scattered, so that, in cross section, the xylem and phloem occurred in irregular patches rather than in one continuous column.

Cortical cells in some infected roots contained one to several thin cross walls and were larger than in check roots. In many infections, a periderm-like formation developed from the outer layers of the cortex on the infected side of the root (Fig. 2, F), even though the nematode was imbedded several cells beneath the surface. It was also observed that in infections by a single female, cell proliferation occurred only on that side of the root in which the nematode was imbedded.

Gall development induced by *M. hapla* was similar to that induced by the other two species tested; extensive lateral roots and root primordia were, however, developed from the cortex and pericycle. Although cortical and stelar parenchyma cells proliferated extensively, cortical cells showed little hypertrophy. This lack of increase in cell size probably accounts for the smaller gall size characteristic of *M. hapla* infected roots.

It is evident that gall formation induced by *Meloidogyne* spp. is similar in all crops which have been studied. Differences in gall size in gardenia infected with

different species of *Meloidogyne* is dependant upon the extent of cellular hypertrophy. Formations of thickenings of portions of root parenchyma cell walls in root-knot of gardenia, rose, and soybean have not been reported in other plants studied.

RÉSUMÉ

Histopathologie de Gardenia infecté par trois espèces de Meloidogyne.

Les auteurs n'ont observé aucune différence dans le développement des galles sur Gardenia causées par les nématodes des racines *Meloidogyne incognita incognita* et *M. incognita acrita.* Des cellules géantes, du xylème anormal, ainsi que de l'hypertrophie et de l'hyperplasie corticales et du stèle ont accompagné toutes les infections. Les cellules corticales de certaines racines infectées étaient pourvues d'un à plusieurs parois transversales minces.

Au côté infecté de beaucoup de racines s'est développé des couches extérieures du cortex un tissus ressemblant à un périderme.

Un épaississement irrégulier des parties des parois cellulaires fut parfois observé aux environs des cellules géantes renfermant des groupes de cellules parenchymateuses.

Le développement de la galle causée par *M. hapla* était identique, mais les cellules corticales n'étaient que faiblement hypertrophiées et une grande production de racines secondaires fut observée.

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CORRIGENDUM

Nematologica 5 (1960) p. 175 (Paper by W. R. Jenkins: *Paratylenchus marylandicus* n. sp.): In the first line of the description of the female, read 1 μ instead of 1 π.

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ÜBER DAS AUFTRETEN FREILEBENDER WURZELNEMATODEN IN RHEINISCHEN BÖDEN UNTER BESONDERER BERÜCKSICHTIGUNG DER STANDORTVERHÄLTNISSE

VON

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Seit einigen Jahren kennt man als Ursache für manche Pflanzenschäden, die früher als „Müdigkeit" bezeichnet wurden, die grosse Gruppe der freilebenden Wurzelnematoden. OOSTENBRINK (1956) rechnet mit einem jährlichen Ertragsverlust von 10-20% an Feldfrüchten auf alten Kulturböden. Bei eingehenden Untersuchungen in Holland fanden OOSTENBRINK (1954) und OOSTENBRINK, S'JACOB & KUIPER (1956) am häufigsten Vertreter der Gattungen *Hoplolaimus* (*Rotylenchus*), *Paratylenchus, Pratylenchus* und *Tylenchorhynchus.* Von diesen kommen in einem Kulturboden meist mehrere Arten der verschiedenen Gattungen vor (OOSTENBRINK 1956, OOSTENBRINK, S'JACOB & KUIPER 1956). OOSTENBRINK (1954) fand in Sandböden vor allem *Pratylenchus pratensis* (De Man) [2]) und *P. penetrans* (Cobb), in Tonböden besonders *P. minyus* (Sher & Allen).

Im Sandboden eines Betriebes stellten OOSTENBRINK, S'JACOB & KUIPER (1956) *Pratylenchus pratensis* an Roggen in weit grösseren Mengen als an Rüben und Kartoffeln fest. Auf einer alten, umgebrochenen Grünlandfläche nahmen im Laufe einer Vegetationsperiode *Pratylenchus* und *Tylenchorhynchus* an Hafer, *Paratylenchus* an Rüben und *Rotylenchus* an Kartoffeln besonders zu.

In Tonböden verursachten *Pratylenchus-, Paratylenchus-* und *Tylenchorhynchus*-Arten bei Weizen und Gerste Ertragsrückgänge. Weiterhin förderten hier Hafer und möglicherweise Rotklee *Pratylenchus,* während nach Lein, Erbsen, Kartoffeln und Rüben die Populationsdichte dieses Schädlings abnahm. Auch im Grünland an Gräsern und Klee trat *Pratylenchus pratensis* auf; in neuester Zeit fand OOSTENBRINK (1959) in Grünland ausserdem *Tylenchorhynchus dubius* (Bütschli) und verschiedene *Rotylenchus*-Arten sowie eine oder mehrere *Criconemoides*-Arten.

In mehreren Gebieten Hollands verursachte eine *Paratylenchus*-Art erhebliche Ertragsminderungen an Möhren. Nach vorläufigen Feststellungen OOSTENBRINK's (1954), der bis zu 4000 Älchen in 200 ccm Boden fand, dürfte die kritische Befallszahl für Schäden zwischen 600 und 1000 *Paratylenchus* in 200 ccm Boden

[1]) Heutige Adresse: Farbenfabriken Bayer AG., Pflanzenschutzwiss. Abt., Leverkusen, Deutschland.

[2]) *Pratylenchus crenatus* Loof, 1960.

liegen. WEISCHER (1957), der in Westdeutschland starken Kümmerwuchs bei Möhren durch *Paratylenchus* feststellte, gibt eine kritische Befallszahl von 1500-2000 *Paratylenchus* in 250 ccm Boden an.

Nicht immer ist eine hohe Populationsdichte parasitischer Nematoden für das Auftreten von Schäden verantwortlich. Baumschulgewächse und Kartoffeln, die durch *Pratylenchus penetrans* stark geschädigt werden können (OOSTENBRINK 1956, OOSTENBRINK, s'JACOB & KUIPER 1957), förderten diese Nematodenart bedeutend geringer als Getreidearten, die keine Schäden aufwiesen.

Im Gegensatz zur amerikanischen und holländischen Literatur liegen über freilebende Wurzelnematoden in Deutschland bisher nur wenige Berichte vor (u.a. GOFFART 1951, WEISCHER 1957, REUVER 1958).

Parallel zu den holländischen Feststellungen sollte deshalb das mengen- und gattungsmässige Vorkommen freilebender Wurzelnematoden an verschiedenen Kulturpflanzen in rheinischen Böden untersucht werden. Zusammenhängend damit war festzustellen, ob auch hier bei verschiedenen Fruchtfolgen eine Zunahme oder ein Rückgang der Parasiten zu beobachten ist. Die Untersuchungsergebnisse eines Fruchtfolgeversuches sollten als Grundlage für die Beurteilung der in drei Betrieben Nordrheins gefundenen Werte dienen.

METHODIK

a) *Bisherige Methoden zur Gewinnung der Nematoden aus Bodenproben*

Über die gebräuchlichsten Methoden ist mehrfach zusammenfassend berichtet worden (OOSTENBRINK 1955, SEINHORST 1956, GOFFART 1959). Von diesen kamen für meine Untersuchungen die Trichtermethode nach Baermann, die mit einer zu grossen Schichtdicke arbeitet, ebenso wenig wie die zeitraubende Sieb- und Dekantiermethode nach COBB (1918) und die aus beiden Verfahren kombinierte Methode von CHRISTIE & PERRY (1951) in Betracht. Auch die direkte mikroskopische Untersuchung des Bodens nach STÖCKLI (1943) ist sehr zeitraubend (OOSTENBRINK 1955) und arbeitet ausserdem mit einer zu geringen Erdmenge je Probe. Mit der Spülkanne von OOSTENBRINK (1954) und den Extraktionsapparat von SEINHORST (1956) werden unter holländischen Verhältnissen 80-90% der Nematoden aus dem Boden gewonnen. Mit beiden Methoden werden zunächst alle in der Erde befindlichen wandernden Nematoden ausgespült und aufgefangen. Bei der nach dem Spülvorgang folgenden Trennung von Nematoden und Schmutzpartikeln auf Wattefiltern bleiben jedoch die inaktiven Älchen auf diesen zurück und entgehen der Auszählung. Deshalb bemühte ich mich um eine Methode, mit der bei geringerem Zeitaufwand ohne fremde Mithilfe nebeneinander viele Bodenproben aufbereitet und ausgezählt werden konnten. Einen guten Ansatz versprach eine von REUVER (1958) benützte Schalenmethode, mit der sie bei *Paratylenchus*-Untersuchungen in schweren Böden bessere Ergebnisse als mit der Oostenbrink'schen Spülapparatur erzielte. Um mit einer geringeren Schichtdicke zu arbeiten, wandelte ich diese Methode ab.

b) *Eigene Methode*

Von Kunststoffrohr (PVC) mit 152 mm innerem Durchmesser wurden 12 mm hohe Ringe abgeschnitten, auf deren eine Seite „Saran"-Siebgewebe (0,90 x 0,75 mm Maschenweite) straff aufgeklebt wurde, so dass eine Siebauflagefläche von 181 qcm entstand.

Eine Erdmenge von 50 ccm ergibt auf ihr eine 2,8 mm dicke Schicht. Um den Einfluss der Schichtdicke auf die Zahl der aus dem Boden auswandernden Nematoden festzustellen, wurden auf die Auflagefläche 25 bzw. 50 bzw. 75 bzw. 100 ccm Boden aufgetragen, deren Schichtdicken etwa 1,4 bzw. 2,8 bzw. 4,2 bzw. 5,5 mm betrugen. Die Ergebnisse der Auszählungen sind in Tabelle I zusammengestellt.

Tabelle I

Aus humosem Sand- und Lehmboden ausgewanderte Nematoden bei verschiedenen Schichtdicken

Gattungen		T.		Prat.		Para.		a. Tyl.		S.	
Schicht-dicke in mm	Boden-art	$\bar{x}$	%	$\bar{x}$	%	x	%	$\bar{x}$	%	$\bar{x}$	%
1,4	Sand	194	58	291	61	45	80	46	46	623	51
	Lehm	114	65	254	53	78	45	64	79	966	61
2,8	Sand	335	100	475	100	56	100	100	100	1230	100
	Lehm	175	100	479	100	174	100	81	100	1579	100
4,2	Sand	398	119	543	114	77	138	128	128	1589	129
	Lehm	156	89	536	112	181	104	128	158	2030	129
5,6	Sand	417	124	601	127	119	212	148	148	1798	146
	Lehm	136	78	733	153	189	109	123	152	2768	175

T.: *Tylenchorhynchus,* Prat.: *Pratylenchus,* Para: *Paratylenchus,* a. Tyl.: andere *Tylenchiden,* S.: Saprozoische Nematoden.

$\bar{x}$ = Mittelwerte aus je vier Wiederholungen.

% = Zahl der Nematoden in Prozent, auf jeweils 2,8 mm Schichtdicke des Bodens in den Sieben berechnet.

Die Menge der ausgewanderten Nematoden nahm mit steigender Schichtdicke ab. Die Werte bei 2,8 mm Erde unterschieden sich nicht wesentlich von denen bei 1,4 mm, wurden aber bei grösseren Schichtdicken für eine Auswertung zu ungenau. Zur Befreiung der Suspensionen von Erdpartikeln wurden auf jedes Sieb vor Einfüllen der Bodenprobe zwei Milchwatteschеiben von 160 mm Durchmesser gelegt. Die Siebe wurden dann in emaillierte Schüsseln mit eingesenktem Boden gesetzt. Der Abstand vom Siebgewebe zum Boden der Schüsseln betrug 6-9 mm. Die Schüsseln wurden mit Wasser soweit aufgefüllt, dass dieses die Erdoberfläche gerade erreichte. 72 Stunden blieben die Siebe in den Schüsseln.

Aus Lehmboden waren nach 24 Stunden 628 (= 84,7 %) der insgesamt 741 im Wasser aufgefangenen Tylenchiden ausgewandert, nach 48 Stunden weitere 93

(= 12,6 %), nach 72 Stunden die restlichen 20 (= 2,7 %). Während die meisten *Pratylenchus* und *Paratylenchus* innerhalb von 24-48 Stunden die Siebe durchwandert hatten, benötigten grosse Nematoden wie *Tylenchorhynchus macrurus* und *Rotylenchus* hierzu 48-72 Stunden.

Zur Bestimmung der quantitativen Ausbeute wurde in 15 Wiederholungen eine bestimmte Zahl von *Pratylenchus, Paratylenchus* und *Tylenchorhynchus* sterilem Lehmboden zugesetzt, von denen durchschnittlich 85 % durch die Siebe auswanderten.

Die Suspension jeder Schüssel wurde nach Entfernung der Siebe auf 100 ccm aufgefüllt und mittels einer Membranpumpe mit Luft durchgewirbelt. Mit einer Pipette wurden dann 2 x 10 ccm in eine Petrischale mit eingesetztem Quadratnetz nach REUVER (1958) gefüllt. Jeder Zählschale wurde eine Prise „Pril" zugesetzt, damit an der Wasseroberfläche schwimmende Nematoden sich absetzten und nicht der Zählung entgingen.

Im allgemeinen zählte ich bei Tageslicht unter dem Binokular mit 50-facher Vergrösserung aus, verwendete aber bei ungünstigen Lichtverhältnissen eine Beleuchtungsanlage nach SIMON (1956). Von jeder Bodenmischprobe wurden drei Wiederholungen angesetzt. Die Zählergebnisse der drei Teilproben wichen im allgemeinen (Mittelwerte aus 12 Mischproben) bei *Tylenchorhynchus* um 23 %, bei *Pratylenchus* um 21 %, bei *Paratylenchus* um 21 % und bei saprozoischen Nematoden um 19 % voneinander ab. Die Zählergebnisse bezog ich auf die Gesamtmenge von 100 ccm Erde.

FELDVERSUCH KLEIN ALTENDORF

Zur Beobachtung der Populationsentwicklung freilebender Wurzelnematoden bei verschiedenen Fruchtfolgen legte ich im Frühjahr 1956 auf dem Versuchsfeld Klein Altendorf bei Meckenheim (Kreis Bonn) einen Feldversuch an. Die dafür ausgewählte Fläche war letztmalig 1953 mit Stallmist gedüngt worden; die Versuchsfrüchte wurden ausschliesslich mit Mineraldünger versorgt. Der tiefgründige Lösslehm hatte 1956 einen Humusgehalt von 2,76 %. Vorfrucht vor Versuchsbeginn war 1955 Hafer. 1956 wurden nebeneinander in zweifacher Wiederholung 7 Parzellen von 21 × 3 m mit Hafer, Sommerweizen, Zuckerrüben, Kartoffeln, Möhren, Wirsingkohl und Markerbsen bestellt. 1957 wurden quer zu den 21 Langparzellen auf vier 5 m breiten Streifen Sommerweizen, Zuckerrüben, Kartoffeln und Möhren angebaut, so dass sich die aus Abbildung 1 ersichtliche Anordnung ergab.

1958 wurde dieselbe Bebauung wie 1956 gewählt. Zwischen dem 1.8.57 und dem 15.12.58 untersuchte ich drei- bis viermal den Boden auf seinen Nematodengehalt. Dabei wurden je Parzelle von 15 qm 30 Einstiche zu einer Mischprobe vereinigt. Zur Ausschaltung von Randwirkungen blieb ein 50 cm breiter Randstreifen ausgespart. Im August 1957 untersuchte ich alle Einzelparzellen der zweiten Wiederholung, späterhin nur noch sechs Einzelparzellen in dreifacher

Wiederholung (s. Abb. 1, schraffierte Parzellen), da die Untersuchung aller Parzellen zum gleichen Termin arbeitstechnisch nicht möglich war.

Die in den Abbildungen 2 a-c dargestellten Zählergebnisse lassen die unterschiedliche Populationsentwicklung freilebender Wurzelnematoden deutlich erkennen.

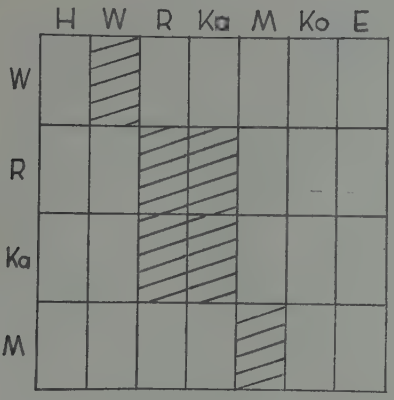

Abb. 1. Lageplan des Feldversuchs Klein Altendorf. H: Hafer; W: Sommerweizen; R: Zuckerrüben; Ka: Kartoffeln; M: Möhren; Ko: Wirsingkohl; E: Markerbsen.

Dreimaliger Anbau von Sommerweizen, Zuckerrüben und Möhren führte zu einem starken Besatz mit *Paratylenchus*.

Bei ausschliesslichem Kartoffelbau stieg dagegen die *Pratylenchus*-Population (ca. $^2/_3$ *P. penetrans*, $^1/_3$ *P. pratensis* in beiden Jahren) erheblich an, während *Paratylenchus* nur in geringer Zahl auftrat.

Bei wechselndem Anbau von Zuckerrüben und Kartoffeln änderte sich der Nematodenbesatz im Laufe einer Vegetationsperiode gleichsinnig wie bei der dreimal aufeinander folgenden Kultur der beiden Früchte. Während durch Kartoffeln *Pratylenchus* gefördert und *Paratylenchus* zurückgedrängt wurden, vermehrten sich in den folgenden Zuckerrüben die *Paratylenchus* bei einem gleichzeitigen Rückgang von *Pratylenchus*.

Neben dem Einfluss verschiedener Fruchtfolgen auf den Nematodenbesatz wurden im gleichen Feldversuch auch die jahreszeitlich bedingten Populationsschwankungen untersucht. In Abständen von fünf bis sechs Wochen wurden auf drei Parzellen Bodenproben (4 Einstiche je qm) entnommen. In den Abbildungen 3 a-c sind die Ergebnisse dargestellt. Soweit verfügbar, sind monatliche Niederschlagsmengen und durchschnittliche Bodentemperaturen in 20 cm Tiefe in einer Vergleichskurve dargestellt [1]).

[1]) Die Wetterdaten wurden mir von der benachbarten Versuchsanlage des Instituts für Obstbau der Universität Bonn in Klein Altendorf freundlicherweise überlassen.

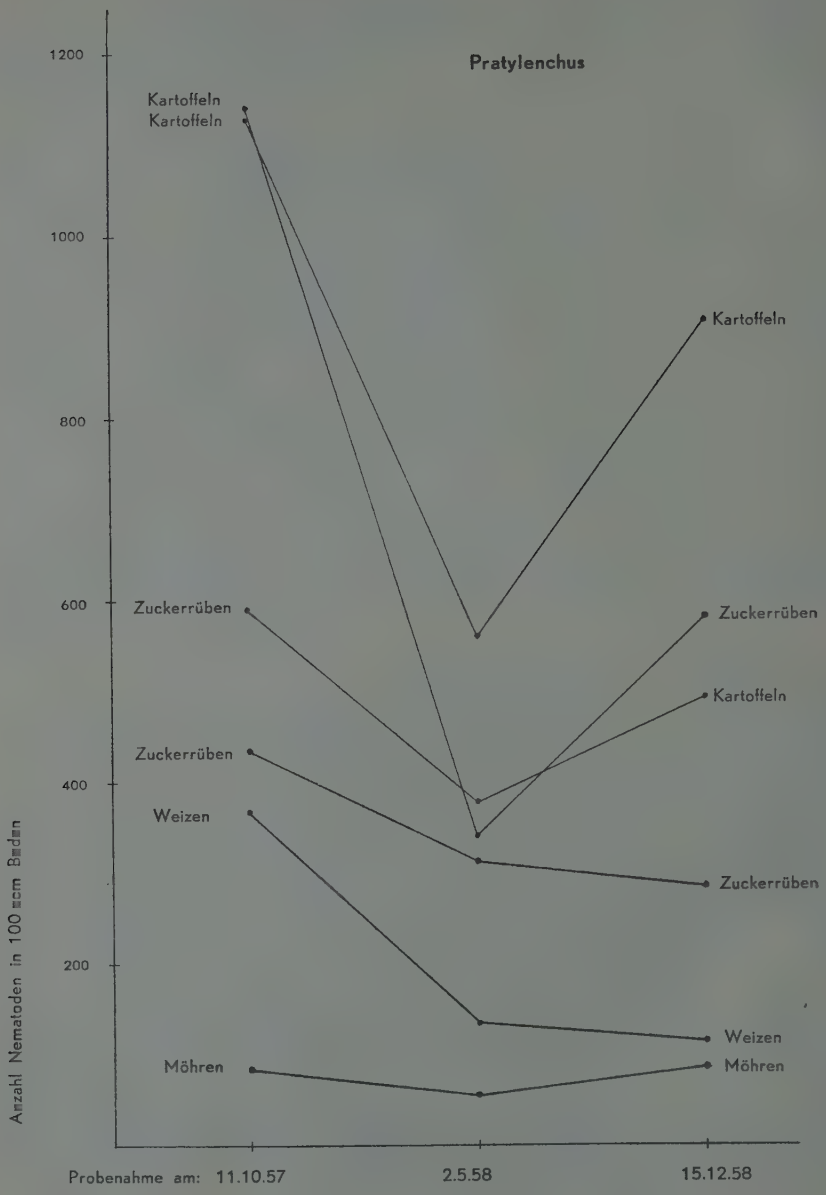

Abb. 2a. Populationsschwankungen von *Pratylenchus* bei verschiedenen Fruchtfolgen.

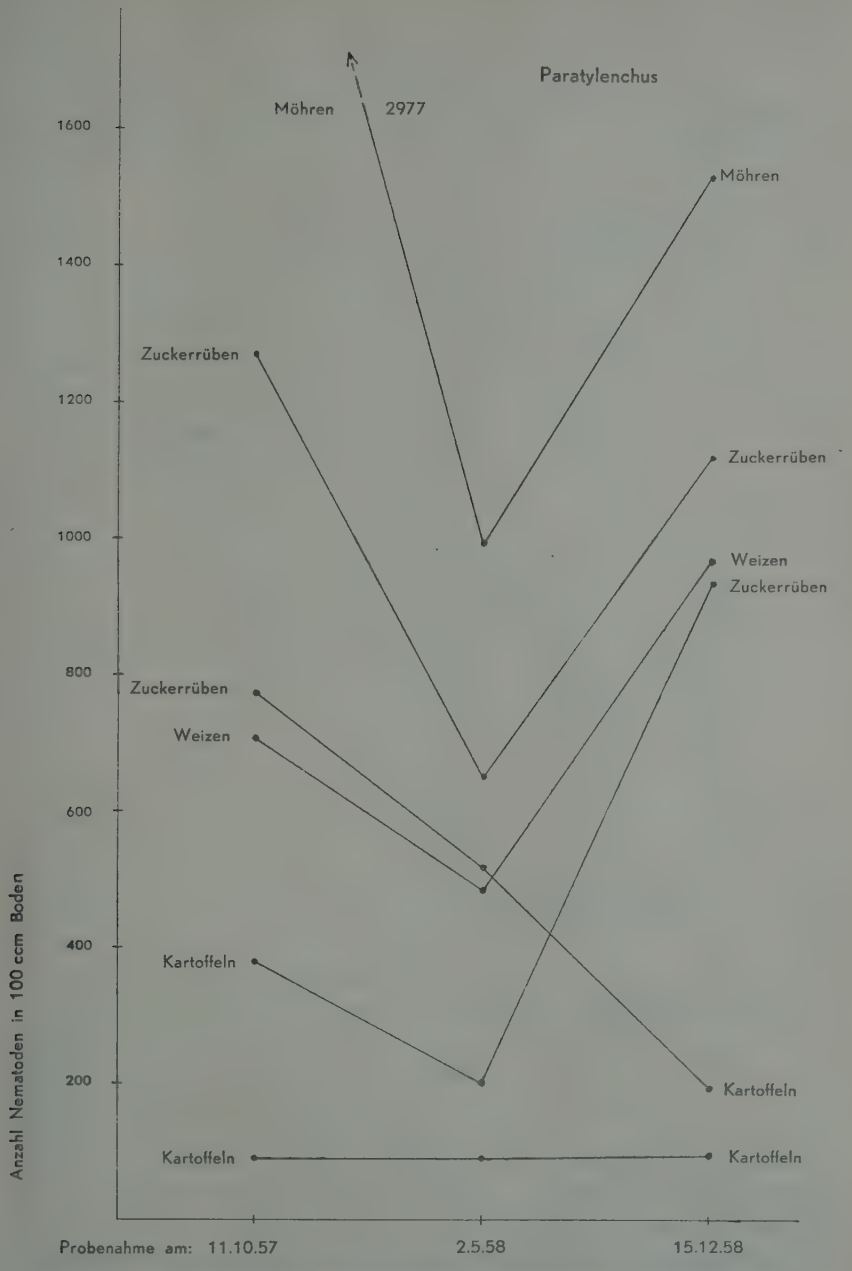

Abb. 2b. Populationsschwankungen von *Paratylenchus* bei verschiedenen Fruchtfolgen

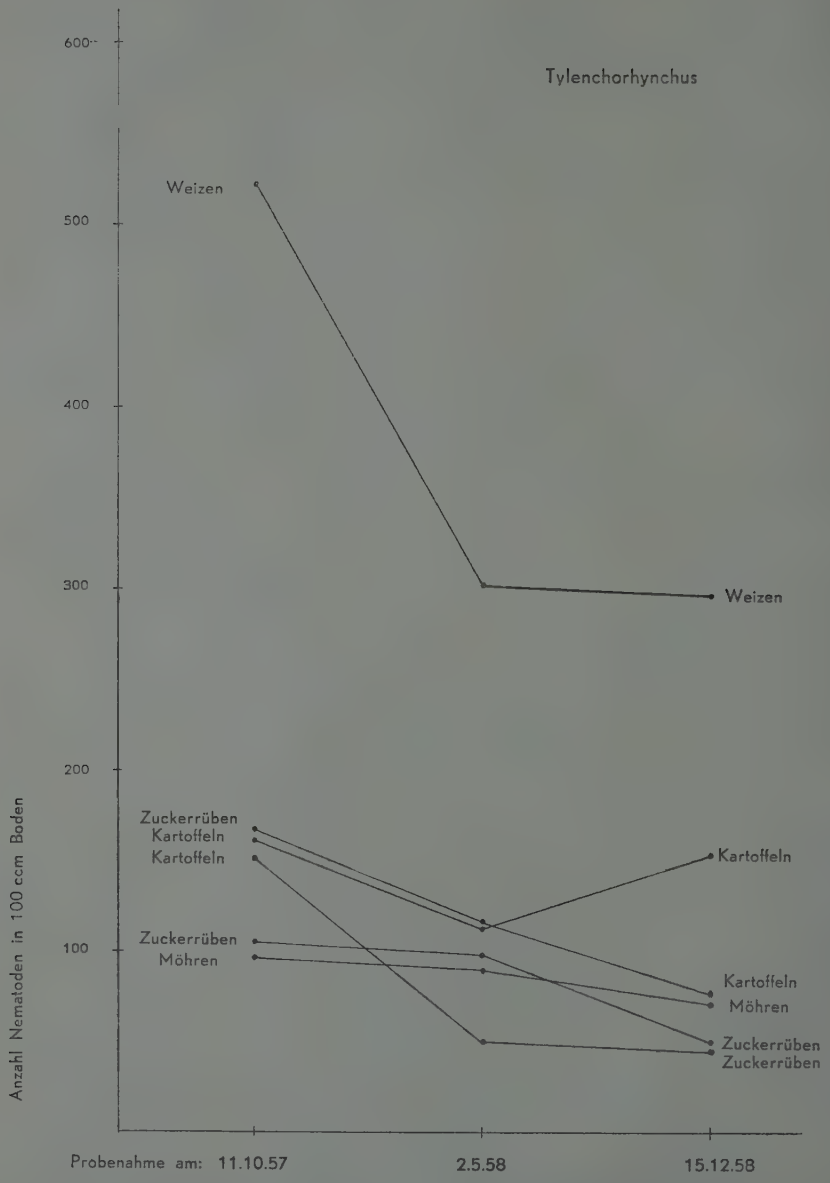

Abb. 2c. Populationsschwankungen von *Tylenchorhynchus* bei verschiedenen Fruchtfolgen.

In der Weizenparzelle (Abb. 3a) erreichte die *Paratylenchus*-Population nach einem Minimum im Juni 1958 ein Maximum im August und ging erst im Spätherbst langsam zurück. Nach einem leichten Anstieg im März 1959 nahm sie weiter ab. *Tylenchorhynchus* zeigte ein Maximum im Juli 1958.

In der Möhrenparzelle (Abb. 3b) ging die *Paratylenchus*-Population nach einem

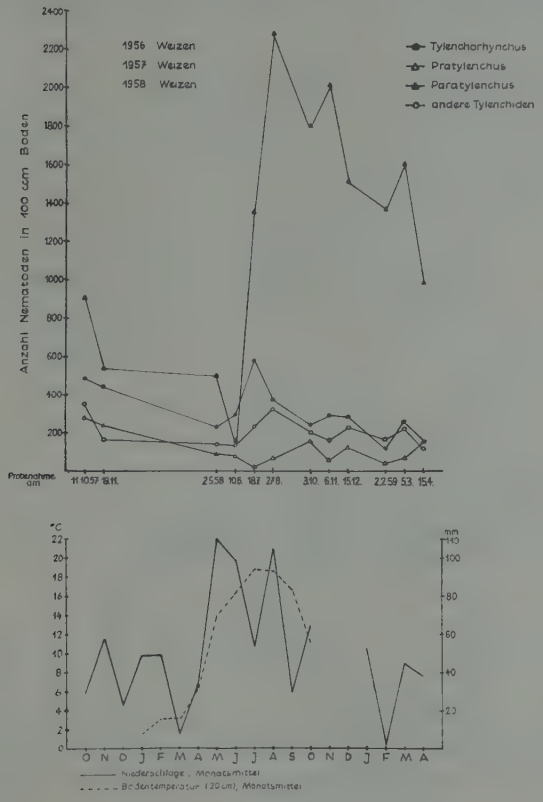

Abb. 3a. Jahreszeitliche Populationsschwankungen freilebender Wurzelnematoden bei dreijährigem Weizenanbau. Niederschläge und Bodentemperaturen von Oktober 1957 bis April 1959.

Höchststand im Herbst 1957 fortlaufend bis zu einem Minimum im Juli 1958 zurück, um dann erneut zu einem Maximum im November anzusteigen. Im August hatte sie bereits das vierfache des Julibestandes erreicht.

In der Kartoffelparzelle (Abb. 3c) ging die *Pratylenchus*-Population (ca. 20 % *P. pratensis*, 80 % *P. penetrans*) von 1200 je 100 ccm Boden im Oktober 1957 auf 550 je 100 ccm im Juni/Juli 1958 zurück, um im August stark anzusteigen und nach vorübergehender geringer Abnahme bis Oktober im November ihr Maximum zu erreichen und dann wieder etwa auf den Juni-Stand abzusinken. Die anderen hielten

sich durchweg etwa auf der gleichen Höhe. Nur *Paratylenchus* liess ein Maximum im Oktober erkennen.

ERHEBUNGEN IN LANDWIRTSCHAFTLICHEN BETRIEBEN VON NORDRHEIN

Um den Einfluss unterschiedlicher Bodenarten, Fruchtfolgen und Witterungs-

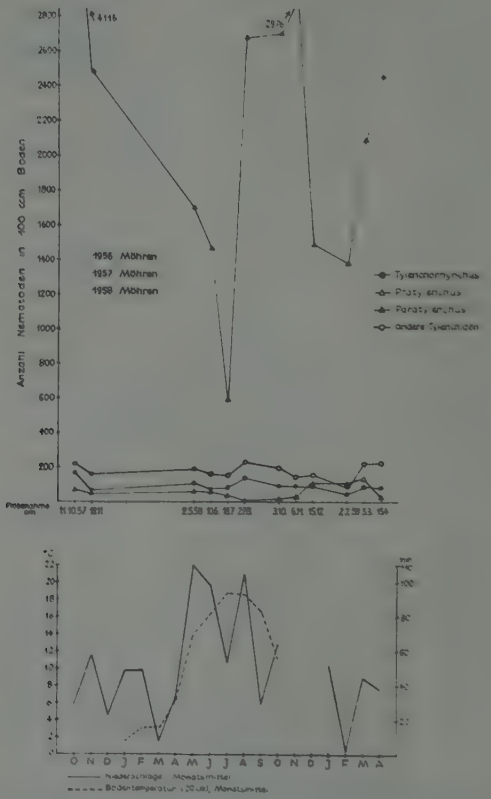

Abb. 3b. Jahreszeitliche Populationsschwankungen freilebender Wurzelnematoden bei dreijährigem Möhrenanbau. Niederschläge und Bodentemperaturen von Oktober 1957 bis April 1959.

verhältnisse auf den Befall durch freilebende Wurzelnematoden zu erfassen, wurden verschiedene Parzellen in drei Betrieben untersucht. In den Jahren 1957 und 1958 wurden drei- bis viermal Bodenproben (400 Einstiche je ha) auf den durchschnittlich 0,5-1,5 ha grossen Parzellen entnommen. Zur Charakterisierung der Bodenarten diente die Bodenübersichtskarte von Nordrhein-Westfalen 1 : 300 000 (MÜCKENHAUSEN & WORTMANN 1953).

Untersuchungen am Niederrhein

Die Parzellen des bei Issum, Kreis Geldern, gelegenen Betriebes bestehen aus tiefgründigem, teils stark kiesigem Sandboden (vermutlich Terrassensand). Im

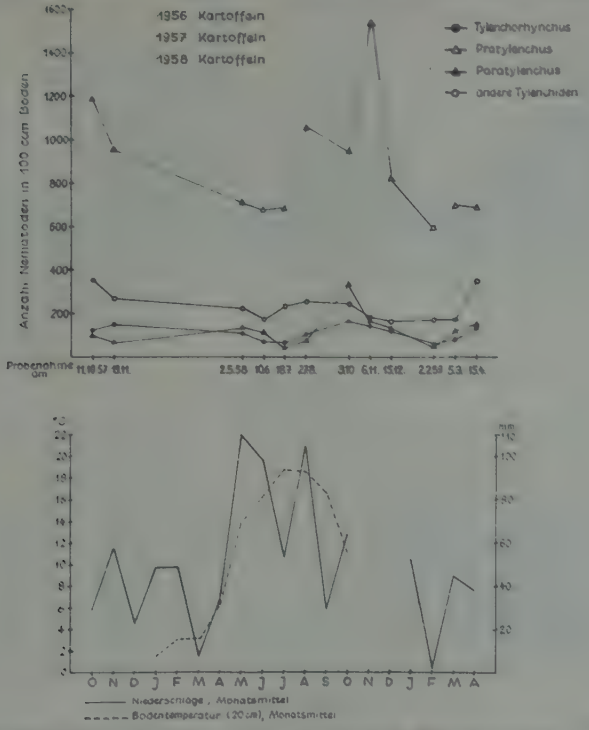

Abb. 3: Jahreszeitliche Populationsschwankungen freilebender Wurzelnematoden bei dreijährigem Kartoffelanbau. Niederschläge und Bodentemperaturen von Oktober 1957 bis April 1959.

Anbauverhältnis stehen Getreide und Inkarnatklee-Raygras-Gemenge den Hackfrüchten gegenüber im Vordergrund. In Tabelle II sind die Anbaupläne der elf untersuchten Parzellen für die Jahre 1956 bis 1958 zusammengefasst.

TABELLE II

Anbaupläne von elf Parzellen bei Issum von 1956-1958

Anbau-jahr	Parzellen 1	2	3	4	5	6
1956	Kartoff.	Roggen	Klee-Gras	Klee-Gras	Roggen	Klee-Gras
1957	Roggen	Klee-Gras	Kartoff.	Hafer	Klee-Gras	Roggen
1958	Klee-Gras	Hafer	Roggen	Klee-Gras	Roggen	Klee-Gras

Anbau-jahr	Parzellen 7	8	9	10	11
1956	Klee-Gras	Hafer-Gerste	Futterrüben	Roggen	Hafer
1957	Roggen	Klee-Gras	Kartoffeln	Futterrüben	Klee-Gras
1958	Klee-Gras	Kartoffeln	Roggen	Hafer	Kartoffeln

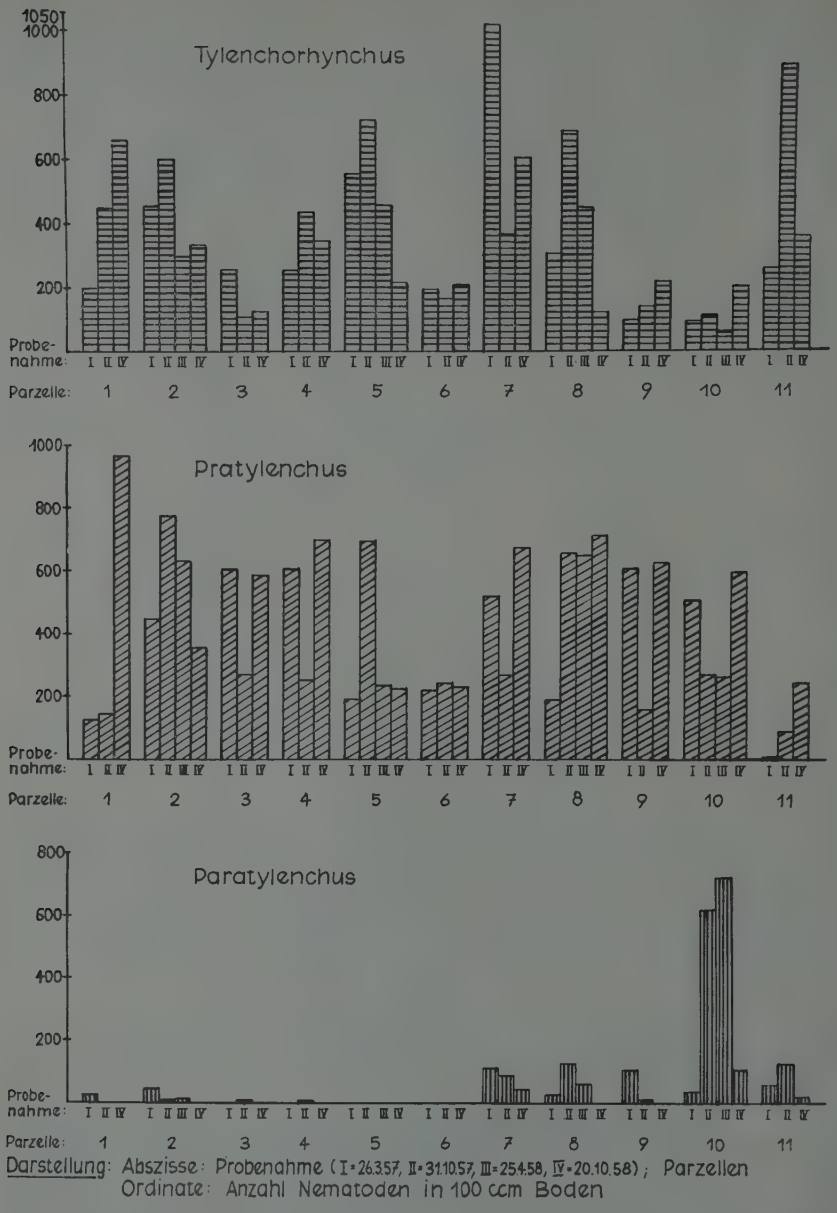

Abb. 4. Untersuchungen des Nematodenbesatzes 1957 und 1958 in elf Parzellen des Betriebes bei Issum.

In drei- bis vierjährigem Abstand wird vor der Hackfrucht mit etwa 300 dz/ha Stallmist gedüngt.

Die Ergebnisse der Bodenuntersuchungen sind in Abbildung 4 dargestellt.

Untersuchungen in der Kölner Bucht

Das Ackerland des Betriebes in Sinthern, Kreis Köln, — tiefgründiger Lösslehm — wird nicht nach festgelegten Fruchtfolgen bebaut. Dreifeldrige Glieder wechseln mit zweifeldrigen ab, mitunter folgen auch zwei Hackfrüchte aufeinander. Im Abstand von drei Jahren erhalten die Parzellen eine Stallmistdüngung von etwa 300 dz/ha.

In Tabelle III sind die Anbaupläne der neun untersuchten Parzellen von 1956 bis 1958 zusammengestellt.

Tabelle III

Anbaupläne von neun Parzellen in Sinthern für die Jahre 1956 bis 1958

Anbau-jahr	Parzellen 1	2	3	4	5
1956	Roggen	Roggen	Roggen	Winterweizen	Zuckerrüben
1957	Rotklee	Kopfkohl	Zwiebeln	Wintergerste	Winterweizen
1958	Kartoffeln	Kartoffeln	Frühkartoff., danach Mais	Kopfkohl	Roggen

Anbau-jahr	Parzellen 6	7	8	9
1956	Winterweizen	Zuckerrüben	Roggen	Kartoffeln
1957	Kartoffeln	Winterweizen	Kartoffeln	Roggen
1958	Zuckerrüben	Klee-Gras	Futterrüben	Zuckerrüben

Die Abbildung 5 gibt die Ergebnisse der Bodenuntersuchungen wieder.

Untersuchungen in der Voreifel

Die landwirtschaftliche Nutzfläche des Betriebes in Kirchheim, Kreis Euskirchen gehört zwei verschiedenen Bodenarten an. Die Parzellen 1-4 bestehen aus Lösslehm über steinigem, feinsandigem Lehm, übergehend in Gestein. Auf den Parzellen 5-7 liegt ein flach- und mittelgründiger, steiniger schwerer Lehm vor; Parzelle 5 hat eine starke, Parzelle 7 eine mittlere Hangneigung.

Der Feldbau des Betriebes steht im Zeichen der Vierfelderwirtschaft; auf eine Hackfrucht, meist Rüben, folgt dreimal Getreide. In Tabelle IV sind die Anbaupläne der sieben untersuchten Parzellen von 1956 bis 1958 zusammengestellt.

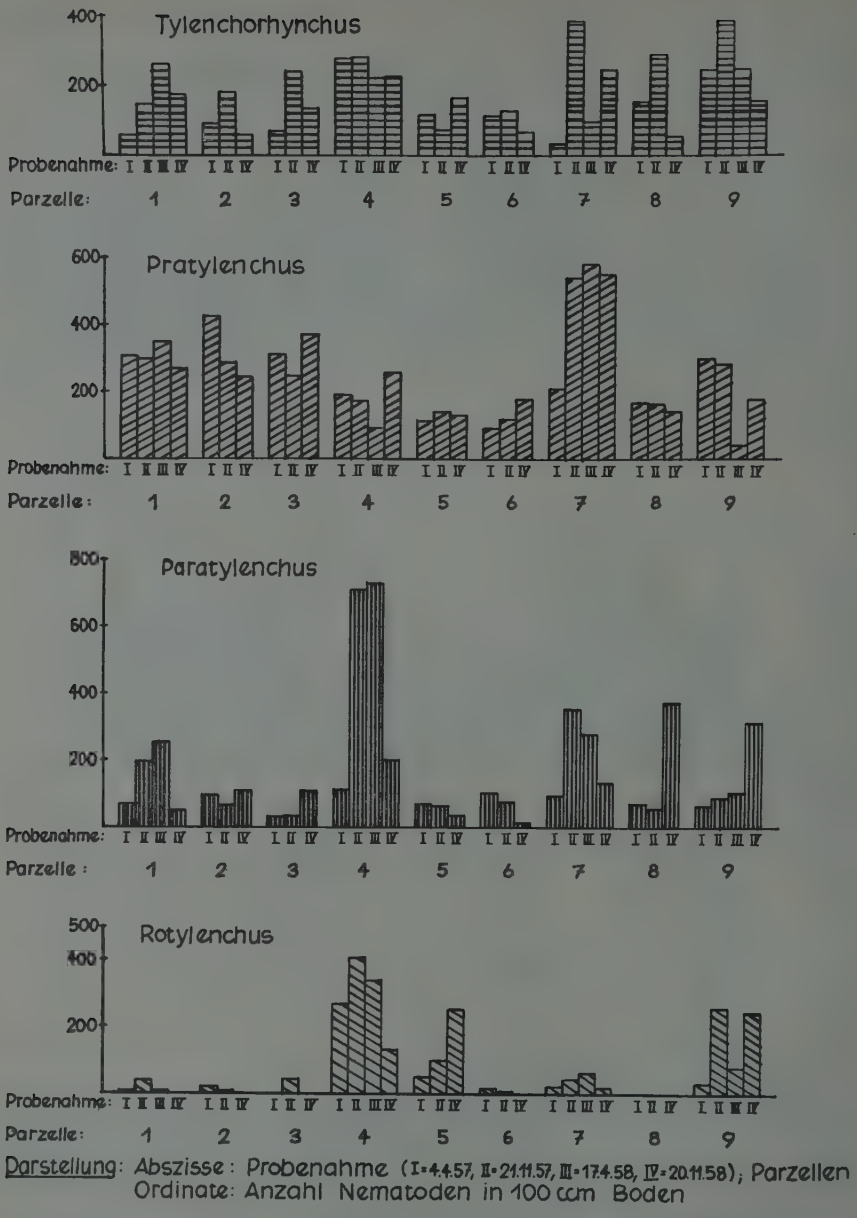

Abb. 5. Nematodenbesatz 1957 und 1958 in neun Parzellen in Sinthern.

TABELLE IV

Anbaupläne von sieben Parzellen des Betriebes in Kirchheim von 1956 bis 1958

Anbau-	Parzellen			
jahr	1	2	3	4
1956	Weide	Roggen	Sommerweizen	Weide
1957	Weide	Hafer	Zuckerrüben	Weide
1958	Weide	Zuckerrüben	Winterweizen	Weide

Anbau-	Parzellen		
jahr	5	6	7
1956	Sommerweizen	Roggen	Winterweizen
1957	Zuckerrüben	Sommergerste	Roggen
1958	Winterweizen	Zuckerrüben	Sommergerste

Vor dem Anbau der Hackfrucht, im Abstand von 4 Jahren, wird der Boden mit Stallmist gedüngt.

Die Ergebnisse der Bodenuntersuchungen sind in Abbildung 6 dargestellt.

Vergleich der drei Untersuchungsergebnisse

Um die in den Abbildungen 4-6 dargestellten Ergebnisse besser miteinander vergleichen zu können, wurden die Populationsveränderungen der freilebenden Wurzelnematoden für die einzelnen Feldfrüchte zusammengefasst. Dabei beschränkte ich mich auf Zucker- und Futterrüben, Kartoffeln, Winterweizen, Winterroggen und Klee-Gras-Gemenge, da diese Früchte 1957 und 1958 in mindestens zwei der drei Betriebe auf mehreren Parzellen angebaut worden waren. In den Abbildungen 7a-e sind nur die Veränderungen des Nematodenbesatzes über 20 % dargestellt, Zu- und Abnahmen unter 20 % blieben unberücksichtigt. Die Populationsschwankungen sind in drei verschiedenen Zeiträumen erfasst worden, vom Frühjahr bis Herbst 1957, vom Herbst 1957 bis Herbst 1958 und vom Frühjahr bis Herbst 1958. Im einzelnen ist jeweils der Herbstwert in Prozenten des Frühjahrswertes ($F \rightarrow H$) bzw. des vorigen Herbstwertes ($H \rightarrow H$) berechnet. Im allgemeinen weichen die Populationsänderungen in den verschiedenen Zeiträumen des Vergleichs nicht wesentlich voneinander ab.

Auf den ersten Blick fallen die sehr unterschiedlichen Verhältnisse in den fünf Feldfruchtbeständen auf.

In den Kartoffelparzellen (Abb. 7b) gingen mit einzelnen Ausnahmen alle freilebenden Wurzelnematoden trotz verschiedener Vorfrüchte zurück.

Im Gegensatz dazu zeigen sich in den Klee-Gras- und Winterweizen-Parzellen durchweg Zunahmen. In den Winterroggen- und Rüben-Parzellen lassen sich jedoch keine einheitlichen Tendenzen feststellen.

Bei Klee-Gras-Gemengebau (Abb. 7e) nahmen *Tylenchorhynchus* und *Pratylen-*

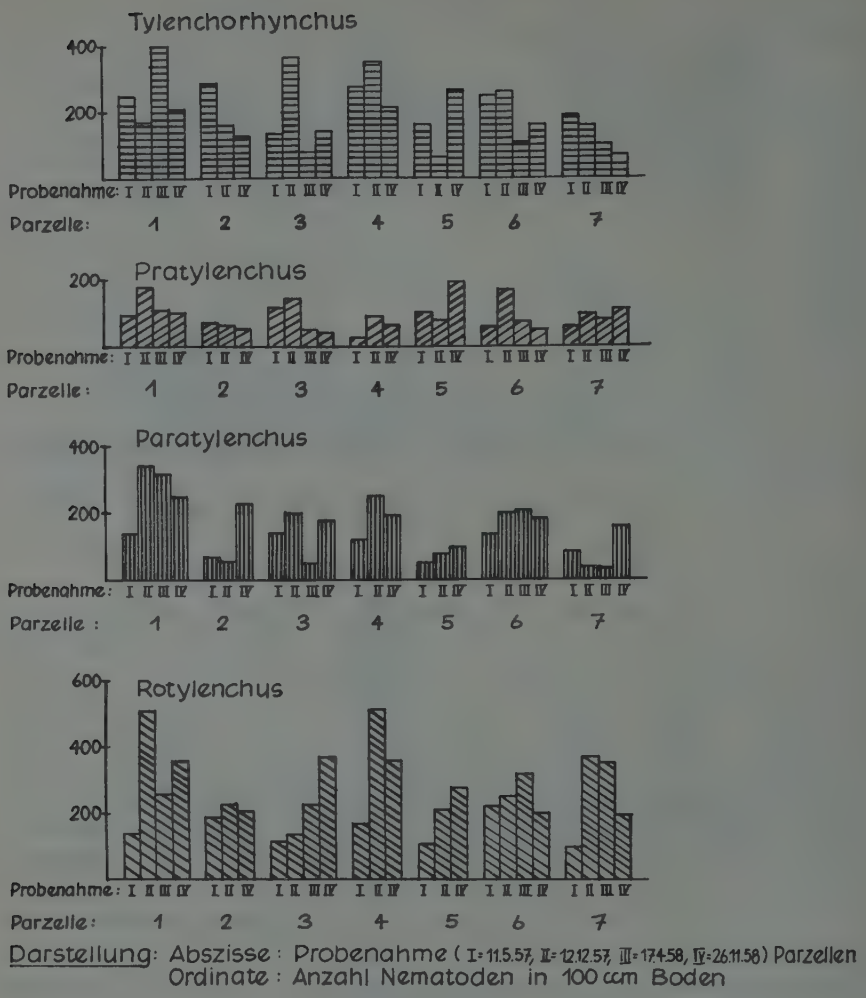

Abb. 6. Nematodenbesatz 1957 und 1958 in sieben Parzellen des Betriebes in Kirchheim.

chus meist auffallend zu, *Paratylenchus* nahm sowohl zu (Vorfrüchte: Sommergetreide) als auch ab (Vorfrüchte: Wintergetreide).

In den Winterweizenparzellen nach Zuckerrüben (Abb. 7c) vermehrten sich *Paratylenchus* und *Rotylenchus* fast durchweg, *Tylenchorhynchus* und *Pratylenchus* dagegen liessen sowohl Zu- als auch Abnahmen erkennen.

Bei Rübenanbau nach verschiedenen Vorfrüchten (Abb. 7a) ging die *Tylenchorhynchus*-Population meistens zurück im Gegensatz zur *Paratylenchus*-Population, die sich überwiegend vermehrte. *Pratylenchus* nahmen sowohl zu als auch ab.

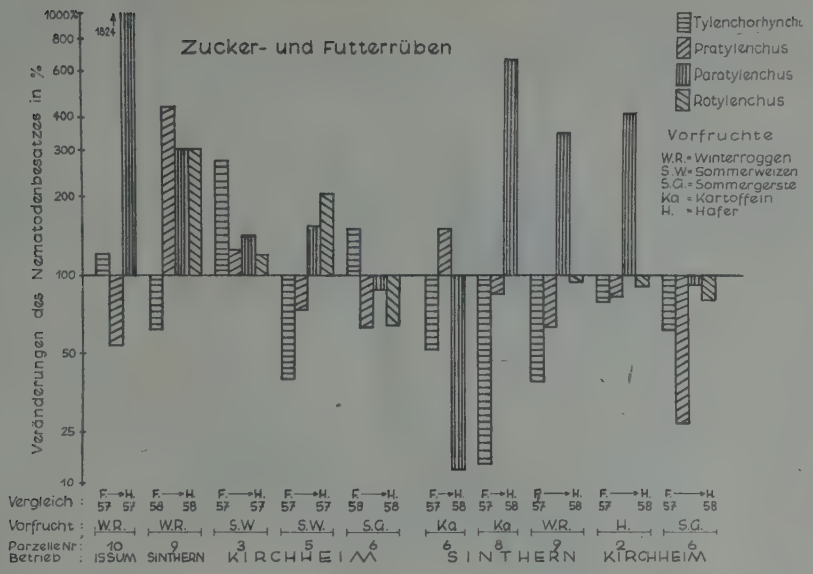

Abb. 7a. Prozentuale Veränderungen des Nematodenbesatzes nach Zuckerrüben- und Futterrübenanbau; Vergleich Herbstbefall (H) gegenüber Befall im vorhergehenden Frühjahr (F) bzw. vorhergehenden Herbst (H).

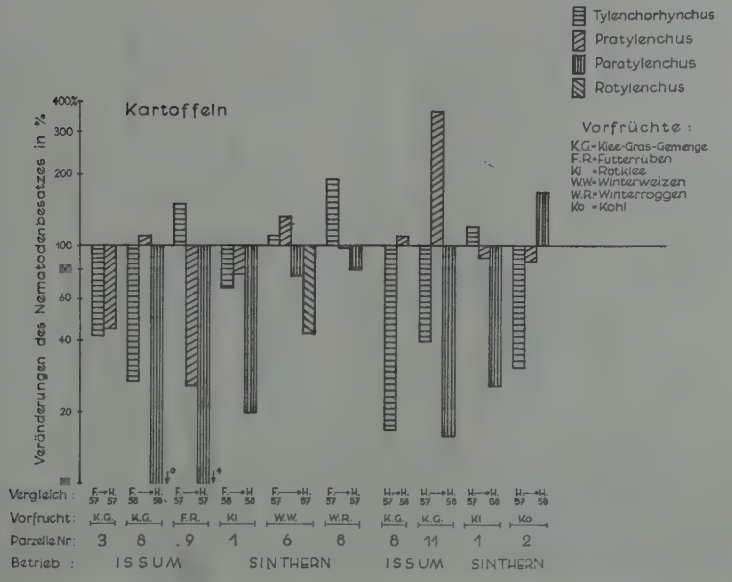

Abb. 7b. Prozentuale Veränderungen des Nematodenbesatzes nach Kartoffelbau vgl. 7a.

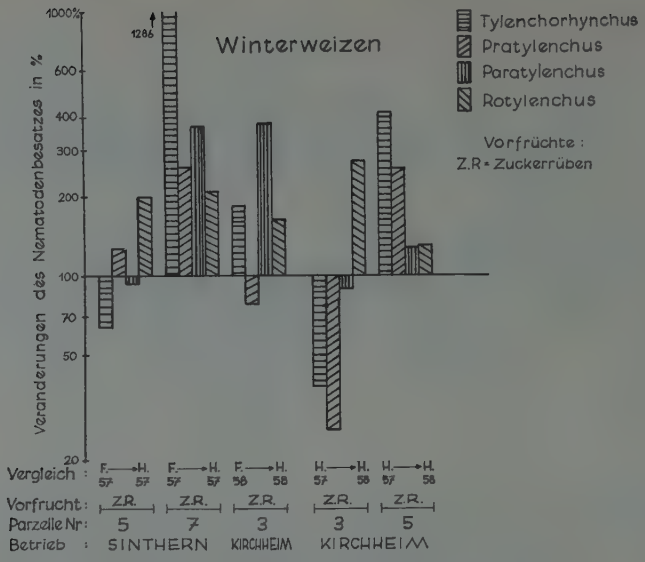

Abb. 7c. Prozentuale Veränderungen des Nematodenbesatzes nach Winterweizenbau vgl. Abb. 7a.

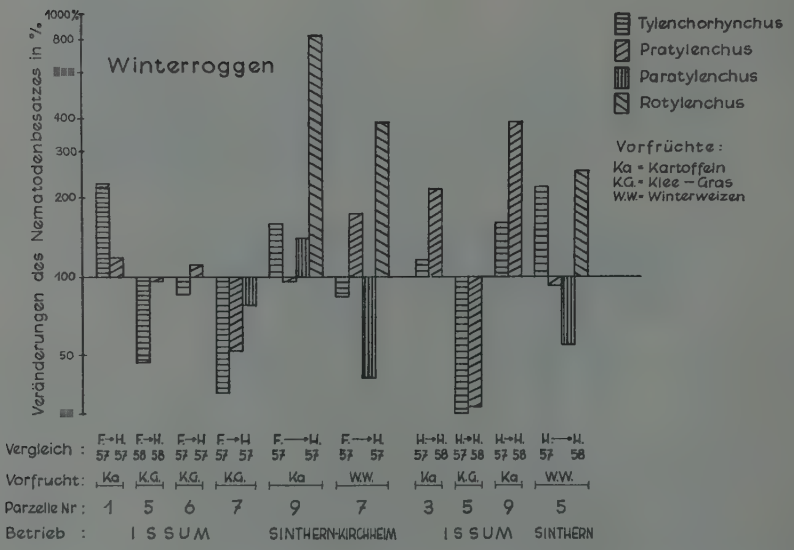

Abb. 7d. Prozentuale Veränderungen des Nematodenbesatzes nach Winterroggenbau vgl. Abb. 7a.

Winterroggenanbau (Abb. 7d) liess *Tylenchorhynchus, Pratylenchus* und *Paratylenchus* gleich häufig zu- und abnehmen. Die Abnahmen traten nach Klee-Gras, von *Paratylenchus* auch nach Winterweizen auf. *Rotylenchus* vermehrte sich in den Parzellen, in denen er vorkam, erheblich.

C. BESPRECHUNG DER ERGEBNISSE

In dem Feldversuch Klein Altendorf wurden unter einheitlichen Bodenverhältnissen (Lösslehmboden) *verschiedene Fruchtarten* mehrmals nach sich selbst

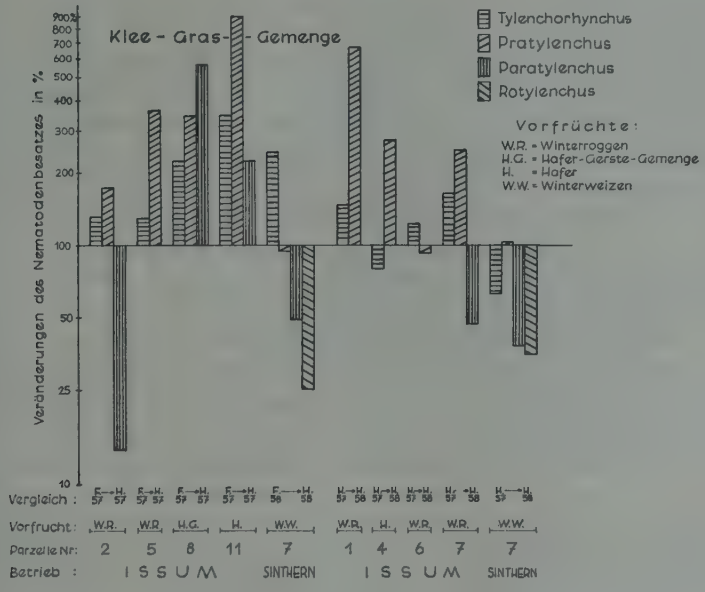

Abb. 7e. Prozentuale Veränderungen des Nematodenbesatzes nach Klee-Gras-Gemengebau vgl. Abb. 7a.

oder nach kurzer Unterbrechung angebaut, um Veränderungen der Nematodenpopulationen zu erhalten, die in geordneten Fruchtfolgen weniger deutlich zu erwarten waren.

Dreijähriger Anbau von Sommerweizen, Zuckerrüben und Möhren führte zu erheblichen Zunahmen von *Paratylenchus,* wie sie OOSTENBRINK, s'JACOB & KUIPER (1956) nach Rüben- und Weizenanbau, OOSTENBRINK (1954) und WEISCHER (1957) nach Möhrenanbau ebenfalls beobachtet haben. Auch der Anstieg der *Pratylenchus*-Population, die zu 65-70% aus *P. penetrans* bestand, nach dreijährigem Kartoffelanbau wurde in Holland beobachtet (OOSTENBRINK 1954, OOSTENBRINK 1956, OOSTENBRINK, s'JACOB & KUIPER 1957). Der in dieser Population 1957 und 1958 ebenfalls festgestellte *P. pratensis,* der sich nicht stark an

Kartoffeln vermehrt, scheint sich an Unkräutern, wie *Poa annua,* erhalten zu haben.

Gegenüber dem Feldversuch Klein Altendorf fällt der im allgemeinen niedrigere Besatz mit freilebenden Wurzelnematoden auf, wie er in den drei landwirtschaftlichen Betrieben festgestellt worden ist. Diese unterschieden sich durch ihre *Bodenarten* grundlegend voneinander. Der Sandboden bei Issum enthielt hauptsächlich die Gattungen *Tylenchorhynchus* und *Pratylenchus,* in mehreren Parzellen auch *Paratylenchus.*

In den Lehmböden von Sinthern und Kirchheim dagegen war der Besatz mit den beiden ersteren Arten geringer; *Paratylenchus* kam in allen Parzellen mehr oder weniger zahlreich vor, daneben fand sich auch *Rotylenchus,* der in Issum und Klein Altendorf fehlte.

Unterschiedliche Schwankungen des Nematodenbesatzes in verschiedenen Böden unter derselben Frucht könnten auf unterschiedlicher Artenzusammensetzung der Gattungen beruhen. Da ich aus arbeitstechnischen Gründen mich auf die Erfassung der Gattungen beschränken musste, konnte ich solche Einflüsse der Bodenart nicht feststellen.

Der Einfluss der *Fruchtart* zeigte sich einmal im Rückgang aller freilebenden Wurzelnematoden nach Kartoffelanbau in allen drei Betrieben mit wenigen Ausnahmen, während in Klein Altendorf *Pratylenchus* zugenommen hatte. Klee-Grasanbau dagegen führte überwiegend zu Zunahmen, wie auch Oostenbrink (1957) erhebliche Zunahmen von *Pratylenchus* an Klee und Gräsern ermittelte. Auch in Winterweizenparzellen überwogen Zunahmen die Rückgänge freilebender Wurzelnematoden, worauf Oostenbrink, s'Jacob & Kuiper (1956) bereits hingewiesen haben. Bei Sommerweizenanbau hatte sich besonders *Paratylenchus* in Klein Altendorf vermehrt. In den Rüben- und Winterroggenparzellen war keine eindeutige Tendenz zu erkennen. In den ersteren fiel der auch in Klein Altendorf beobachtete starke Anstieg von *Paratylenchus* auf. In letzteren gingen nach Vorfrucht Klee-Gras *Pratylenchus* und *Tylenchorhynchus* zurück, während Oostenbrink (1957) gerade auf die ungünstige Vorfruchtwirkung von Klee und anderen Leguminosen durch Förderung bestimmter *Pratylenchus*-Arten hingewiesen hatte.

Wie weit sich meine Feststellungen im einzelnen verallgemeinern lassen, muss weiteren Untersuchungen überlassen bleiben; doch weisen sie darauf hin, dass bei Einhaltung vielseitiger Fruchtfolgen und regelmässiger Humusversorgung die Gefährdung der Fruchtarten durch freilebende Wurzelnematoden im allgemeinen gering sein dürfte.

Neben den Populationsveränderungen im Gefolge des Anbaus verschiedener Fruchtarten wurden in Klein Altendorf auch *jahreszeitlich bedingte Schwankungen* des Nematodenbesatzes beobachtet. 1958 liessen beide dort gefundenen Gattungen (*Pratylenchus, Paratylenchus*) ein Minimum im Frühsommer und ein Maximum im Laufe des Herbstes erkennen, Schwankungen, die möglicherweise mit dem Wachstumsrythmus der Pflanzen in Beziehungen stehen. Zu Beginn ihrer Entwicklung boten diese nur geringe Nährstoffmengen. Im Juni und Juli, zur Zeit der Minima, hielten sich die freilebenden Wurzelnematoden vermutlich haupt-

sächlich in den Wurzeln oder in ihrer näheren Umgebung auf, waren aber nicht im Boden verbreitet. Anders zur Zeit der Ernte. In Sommerweizen und Möhren erreichte *Paratylenchus* sein Maximum kurz vor der Ernte (28.8. bzw. 18.11.58), in Kartoffeln *Pratylenchus* sein Maximum nach der Ernte (10.10.58). Die endoparasitisch lebenden *Pratylenchus* verliessen die Kartoffelwurzeln anscheinend erst nach der Ernte in grosser Zahl, so dass sie bei der anschliessenden Bodenuntersuchung zahlreich gefunden wurden. Ein rascher Rückgang beider Gattungen nach Erreichen des Maximums dürfte sich zwanglos mit dem Fehlen der Wirtspflanzen, mit den Erntemassnahmen und den dadurch verschlechterten Lebensbedingungen erklären lassen.

Die jahreszeitlichen Populationsschwankungen deuteten auch auf Zusammenhänge mit Bodentemperatur und Niederschlägen. In den drei untersuchten Parzellen erreichten *Pratylenchus* und *Paratylenchus* (mit Ausnahme von *Paratylenchus* in der Weizenparzelle) ihr Minimum bei relativ geringen Niederschlägen im Juli, während der Anstieg zum Maximum mit reichlichen Niederschlägen bei hohen Bodentemperaturen einherging. Ähnliche Beziehungen zwischen den Populationsbewegungen von *Paratylenchus amblycephalus* und dem Temperaturverlauf stellte Reuver (1958) fest.

Im Zusammenhang mit den jahreszeitlichen Populationsschwankungen erscheint auch der *Zeitpunkt der Probenahme im Herbst* zur Beurteilung des Einflusses der Feldfrüchte auf den Nematodenbesatz von Bedeutung. Eine Bodenuntersuchung im Oktober 1958 hätte gegenüber einer Mitte Dezember durchgeführten sicherlich wesentlich höhere Werte ergeben, da die Nematoden zu dem ersteren Zeitpunkt ihr Maximum erreichten. Vielleicht lassen sich damit auch die grossen Unterschiede der *Paratylenchus*-Zahlen in den Möhrenparzellen zwischen Oktober 1957 und Dezember 1958 erklären.

Meinem verehrten Lehrer, Herrn Prof. Dr. H. Braun, danke ich herzlich für die Förderung meiner weiteren Ausbildung und alle mir gewährte Hilfe. Ebenso gilt mein Dank Herrn Dozent Dr. V. Moericke für mannigfache Anregungen und Unterstützung bei der Durchführung meiner Untersuchungen.

SUMMARY

1. With a modified "Baermann-method" it was possible to find approximately 85 percent of the mobile free-living root-nematodes in the soil.

2. In a field test at Klein Altendorf (district of Bonn) a considerable increase was obtained in the population of *Paratylenchus* when growing wheat, sugar-beets and carrots three times and of *Pratylenchus* (mainly *P. penetrans*) when growing potatoes three times. Beets following potatoes caused a decrease in *Pratylenchus*, potatoes following beets a decrease in *Paratylenchus*.

3. Observations on the population at Klein Altendorf in 1958 showed, for both *Pratylenchus* and *Paratylenchus*, a minimum in June/July and a maximum between August and November. The increase after the minimum followed high soil temperatures and heavy rains. Except for *Paratylenchus* in the wheat plot the minima were observed in July, the month with relatively little rainfall.

4. In a sandy soil (Issum/district of Geldern) the total population of free-living root-nematodes was composed of *Tylenchorhynchus* and *Pratylenchus* and to a lesser degree of *Paratylenchus*.

In loess-loam (Sinthern/district of Köln) and in stony, partly heavy loam (Kirchheim/district of

Euskirchen) *Rotylenchus* were also present. Potatoes always caused a decrease in free-living root-nematodes whereas clover-grass and winter-wheat almost always caused an increase. No significant tendency could be observed in the winter-rye and sugar-beet plots.

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Eingegangen: April 16, 1960

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PARASITISM OF CRANBERRY ROOTS BY *TETYLENCHUS JOCTUS* THORNE [1])

BY

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A survey of the plant nematodes which inhabit soils of cranberry bogs revealed that *Tetylenchus joctus* Thorne, and another nematode of this genus tentatively identified as *T. productus* Thorne, are associated with the roots of the cultivated cranberry (*Vaccinium macrocarpon* Ait.). Nematodes of this genus were found in 36 bogs of a total of 160 sampled during the years 1958 and 1959 (ZUCKERMAN & COUGHLIN, 1960). The current paper describes feeding on cranberry roots *in vitro* by *T. joctus* and it appears to be the first description of parasitism to higher plants by *Tetylenchus*.

MATERIALS AND METHODS

Stock cultures of *T. joctus* used in these experiments were kept in the greenhouse at 18°-29° C in two pots, each of which contained three rooted cranberry cuttings in a sterilized mixture of equal parts by volume of sand and peat. Forty-two specimens were placed in one pot and 31 in the other. These cultures were sampled 4 times at 2 monthly intervals to observe any increase in the nematode population. Nematodes were extracted from the soil by the technique of CHRISTIE & PERRY (1951) and later by the elutriation method of SEINHORST (1956).

The feeding habits of the nematodes were studied by placing 2-10 specimens in petri dishes which contained 4-8 day old cranberry seedlings which had been grown from seed in a 1 % water agar medium. The medium was firm enough to support the seedlings and allowed free movement of the nematodes.

Generally the roots of the seedlings grew closely appressed to the bottom of the petri plate, so that when the plate was inverted feeding was easily observed under a stereoscopic microscope or under the low powers of a compound microscope. Between observations the plates were kept in the dark. The nematodes appeared to be photophobic and usually moved away from illuminated areas. Very occasionally the light from the microscope lamp did not cause a cessation of nematode feeding. The plates which contained the seedlings and the nematodes were observed for varying periods up to 25 days.

For closer observation, a block of agar which contained a seedling and several

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[1]) Contribution No. 1246, Dept. of Entomology and Plant Pathology, University of Massachusetts, Amherst, Mass.

nematodes was cut from the plate, inverted over a deep well slide, and a cover glass placed over the agar. The slide was then placed in a darkened moist chamber for 24 hours and examined at daily intervals thereafter. In this manner feeding was observed in two cases at magnifications up to 1940 ×.

RESULTS

No specimens of *T. joctus* were found in either pot when sampled 2 and 4 months following inoculation. Six months following inoculation the pot in which 31 nematodes had been inoculated contained approximately 1250 and 2 months later the population was estimated at 3050. The population in the other pot reached 950 at 8 months.

Feeding of a total of 19 nematodes, 15 larvae and 4 females, was observed for varying periods of time. No males were seen to feed. Cranberry roots have no root hairs and the nematodes usually fed near the root tip or in the zone of differentiation, but occasionally they fed nearly 2 mm from the root tip.

Feeding was confined to the epidermal cells of roots submerged in the agar. Nematodes were observed feeding on single cells for periods of time varying from 30 seconds to 7 minutes. Feeding was preceded by a short, probing motion during which the stylet was inserted about one-fourth its length into the cell at the rate of 3-6 times per second. Often prior to feeding the nematode moved and repeated the probing action on another cell. Once feeding commenced the nematode was quiescent. Frequently the nematode assumed a position parallel to the long axis of the root. The rear portion of the body was appressed to the root and the forward portion arched in a semicircle so that the head was almost perpendicular to the root (Fig. 1). The stylet penetrated the cell 2-4 μ or up to one-quarter of its total length. The median esophageal bulb pumped rapidly and the valve of the bulb was seen opening and closing. The cytoplasm of the cell was rapidly ingested and often after one minute a viscous mass of cytoplasm was visible about the tip of the stylet. Shortly thereafter this too was ingested. Apparently a large portion of the protoplasmic content of the cell was ingested. During feeding the stylet did not move. When feeding ceased the nematode moved its stylet back and forth 2 or 3 times, in a short probing motion, prior to moving to another cell.

RÉSUMÉ

Parasitisme de racines de Vaccinium macrocarpon (*"Cranberry"*) *par* Tetylenchus joctus *Thorne.*

Des boutures enracinées de *Vaccinium macrocarpon* se trouvant dans une terre stérilisée furent infectées artificiellement par un nombre déterminé de *Tetylenchus joctus.* Ces nématodes s'étaient fortement multipliés après 6 mois.

La nutrition de ces nématodes fut examinée sur des racines de "cranberry" cultivées sur agar dans des boîtes de pétrie. Le mode de nutrition et le comportement du nématode pendant la nutrition sont décrits et illustrés.

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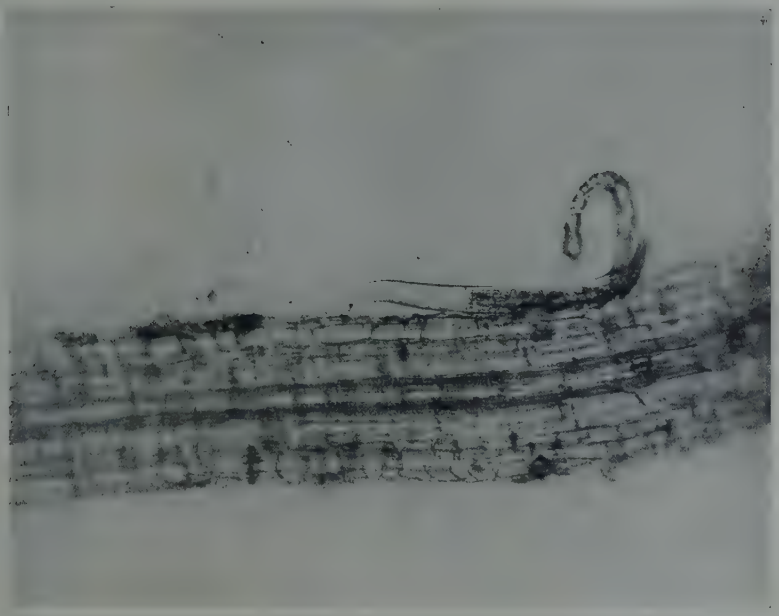

Fig. 1. *Tetylenchus joctus* feeding on a cranberry root *in vitro*. A portion of another nematode is visible to the rear and above the nematode which is feeding. (× 150).

APPARATUS FOR STERILIZING THE AGAR MIXTURE AND MAKING THE LAYERS IN THE BAGS

The agar mixture is put in a small autoclave or pressure cooker, which can withstand a pressure of 2 atmospheres. The vessel (fig. 1, A) is provided with a manometer (fig. 1, c), a safety valve (d), an inlet with air filter (B) (a metal tube densely packed with cotton wool) and an outlet with valve (b). There is also a valve between the air filter and the vessel to prevent agar from entering the former during the sterilisation (fig. 1, a) and one in the air inlet to the air

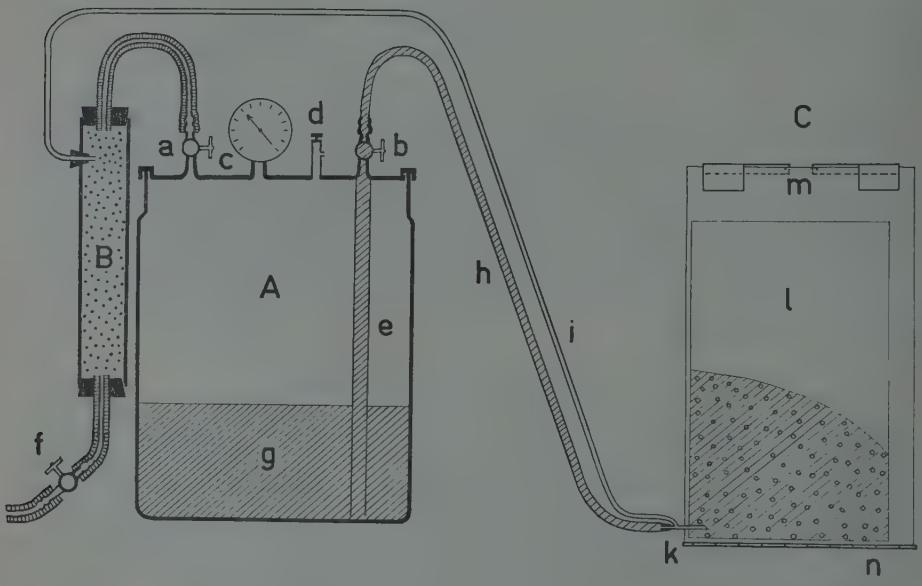

Fig. 1. Diagram of the apparatus, 1/6 natural size. A. Pressure vessel; B. Air-filter with cotton wool; C. Press to keep bags flat; a. valve for air inlet; b. valve for agar outlet; c. manometer; d. safety valve; e. tube for conducting agar to plastic bag; f. valve in pressure line; g. 2½% agar, ½% methylcellulose, nutrient solution; h. tube to injection apparatus; i. tube for injection of air; k. injection apparatus (see fig. 2); l. polyethylene bag; m. clamps for keeping perspex and metal sheet together; n. piano hinge.

filter (f). The outlet is provided with a rubber tube (h) fortified with linen tape to take a pressure of two atmospheres. From the air filter (B) air under pressure can be led through a thin rubber tube (i). To the tubes h and i an apparatus (fig. 1, k, fig. 2) is attached by which agar with small air bubbles can be led into a polyethylene bag (fig. 1, l). On tubes h and i pinchcocks are placed (fig. 2, s). These are connected in such a way that they open simultaneously (see fig. 5) so that agar can pass through h and o into p. If at the same time sufficient pressure is maintained in tube i, air will flow through q (soldered in screwcap r) into the

agar. The end of needle q is narrowed to a very small opening in order to get the right amount of air in comparison to the amount of agar flowing into the plastic bag (1 ml atmospheric pressure for 5-6 ml of agar or 3 ml per second). Sterilisation of apparatus and agar-methyl-cellulose-nutrient solution mixture is done by autoclaving at 120°C for two hours. Valves a and b (fig. 1) are closed before the container with agar mixture and air filter are placed in the autoclave. The tubes h and i are placed in the loops of the pinchcocks. (Sterilizing these tubes when pinched would spoil them). The screwcap r with canula q is unscrewed from tube o. The open end of the latter is plugged with cotton wool. The amount of air delivered by the canula can be measured after sterilizing without interfering with the sterility of tube o. As the fine opening of q (fig. 2) is liable to be clogged, it is checked before use and adjusted if necessary. For the same reason sterile spare canulae ready fixed in screwcaps must be kept at hand in case a canula becomes irreparably clogged. After checking the amount of air delivered by the canula and before replacing it in tube o, it is surface sterilized with burning alcohol. Contamination of the inside of the canula during the manipulations after the autoclaving, is unlikely.

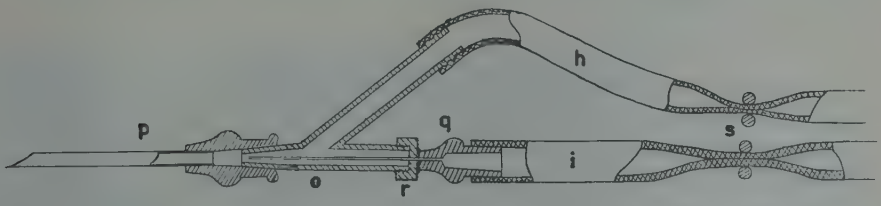

Fig. 2. Apparatus for mixing air and agar, 2/3 natural size. o. Metal tube (fitting into socket of hypodermic needle); p. 2mm hypodermic needle; q. Hypodermic needle adapted for air injection; r. Screwcap for easy replacement of q; s. Two pinchchocks connected so that they open simultaneously; h. Rubber tube conducting agar; i. Rubber tube conducting air.

For cooling and aeration of the agar after sterilisation, the container is shaken gently in a shaking machine, enough to make the agar circulate to bring all of it in contact with air and prevent it from solidifying locally, but not so much that foam is formed, which cannot be used in the bags. During the shaking and the making of the layers in the bags the pressure in the container is kept at 2 atmospheres. When the agar mixture has reached a temperature of about 50°C the making of the layers in the bag can start. The amount of air passing through q is checked, q replaced in o, tubes h and i are replaced in the pinchcocks and valve b is opened.

MAKING THE AGAR LAYERS IN THE BAGS

To make a thin agar layer in a bag the latter is placed in a press made of a piece of stiff metal sheet and a piece of 4 mm perspex sheet of equal size, connected

by a piano hinge (fig. 1, C, n, fig. 5). At the side opposite to the hinge the two plates can be kept together by a clip (fig. 1, m, fig. 5). Near the hinge there is a groove in the perspex just large enough for a 2 mm hypodermic needle. The bag is placed with one corner just under this groove (fig. 6). The corner of the bag in the press, which will lie below the groove in the perspex when the press is closed, is sterilized with alcohol. The hypodermic needle is cleaned with burning alcohol and then inserted in the bag at the sterilized corner (fig. 6). The needle must be well sharpened, otherwise it will stretch the polyethylene before penetration and, instead of slipping between the two layers, it may also pierce the second one. The press is closed and, by opening the pinchcocks, agar with air bubbles is let into the bag (fig. 5). The viscosity and solidifying of the agar between the cold plates prevents the bubbles from ascending quickly so that they stay well dispersed in the agar layer. It is advisible to put the press vertical until the agar has become solid. To prevent micro-organisms from entering the plate, the corner with the puncture is sealed off from the remainder of the bag.

After use, the mixing apparatus (fig. 2) must be cleaned carefully in hot water and the fine canula q blown through and dried. This will keep the aperture of canula q open and free from deformation.

PLANTING SEEDLINGS IN THE AGAR PLATES

Seeds are surface sterilized by treating them for 7 minutes with 0.2 % sublimate containing a spreader and germinated on sterile filterpaper in a Petri dish. Seedlings 0.5 to 1 cm long can be planted. Two cuts (see fig. 7) are made at a right angle near the top of the bag after moistening the area with alcohol for sterilisation of the outer surface. A seedling is then dipped in a saturated solution of bleaching powder (calcium hypochlorite), put on a piece of filter paper for a few seconds and then placed in the agar at the end of the cut, made parallel to the upper edge of the bag, with the cotyledons outside the bag (fig. 7). The cuts are sealed by applying a narrow strip of paraffin wax with low melting point or vaseline-paraffin mixture with a hot needle (fig. 8a). To protect these seals from damage they are covered with strips of adhesive cellophane previously sterilized in ether (fig. 8b). If these strips are put on the unsealed cuts water from the agar often prevents the strips from becoming attached firmly to the polyethylene.

The small opening which remains around the seedling is sealed with some sterile silicone grease.

The bags with growing plants are stored in a slightly slanting position, so as to make all roots grow in one direction against the lower side of the bag where they can easily be observed even with an oil immersion lens.

INOCULATION WITH NEMATODES

Nematodes to be placed in the agar layers are sterilized by putting them in a drop of water containing 0.4 % of streptomycin sulphate (MOUNTAIN, 1959),

H. DEN OUDEN: *Making thin agar layers in polyethylene bags.*

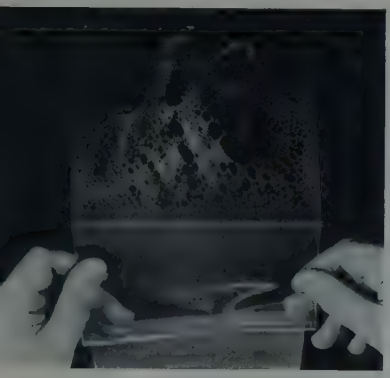

Fig. 3. Removing of air and water from sterilized polyethylene tube.

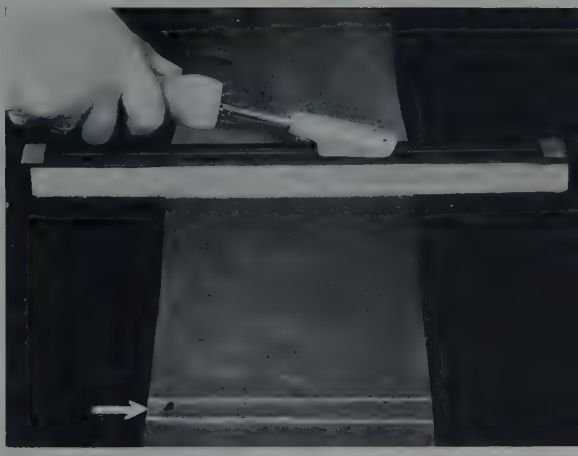

Fig. 4. Sealing apparatus used for cutting polythene tube int closed bags.

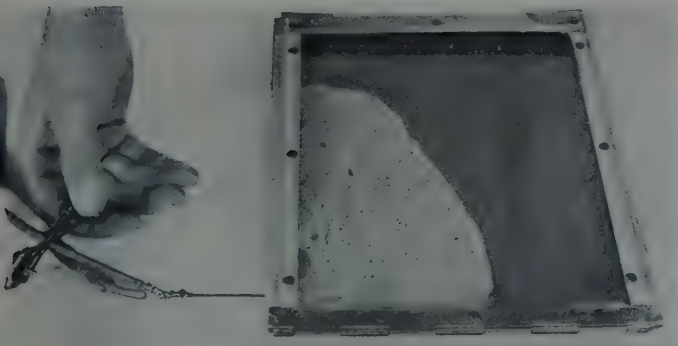

Fig. 5. Press used in filling po- lyethylene bag with mixture of sterile air and agar.

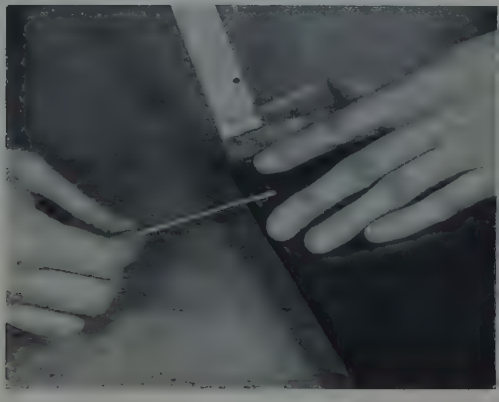

Fig. 6. Inserting 2 mm hypodermic needle into polyethy- lene bag lying in position on open press.

H. DEN OUDEN: *Making thin agar layers in polyethylene bags.*

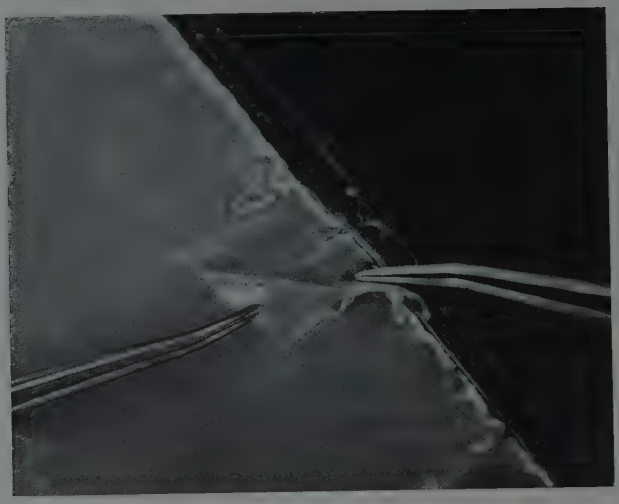

Fig. 7. Inserting a young seedling into a bag.

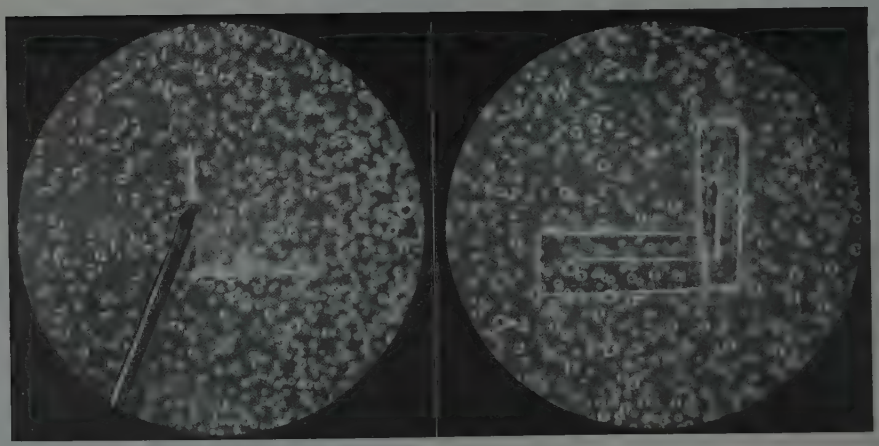

Fig. 8. a) Sealing of cuts in polyethylene with paraffin wax; b) Covering of seal with adhesive cellophane.

for at least ten minutes. After sterilizing an appropriate area of the bag with alcohol, two cuts are made in the polyethylene as described for the planting of seedlings. Some of the agar below the cuts is removed and the sterile nematodes are placed in the air space made in this way. When a number of nematodes are placed close together in the agar, an air space is necessary to keep the nematodes active. The cuts are sealed as described above. Large numbers of nematodes can be transferred rapidly by placing them first in a small drop of water (or streptomycin sulphate solution) on a freshly made layer of paraffin wax in a watch glass (SEINHORST, 1945). Here a drop does not spread out. If the size of the drop is reduced by sucking off water, it can be brought back to an almost spherical shape by blowing against the edge of the water through a pipette. When the drop is made small enough, practically all nematodes can be taken from it in one movement with a stiff bristle or bamboo splint.

WATERING GROWING PLANTS

As soon as the agar begins to shrink along the roots as a result of water consumption by the plant, sterile water is injected through a hypodermic needle. It is advisable to keep the bags flat for a time, otherwise the water may run off to the lowest place. Sterilizing the bag around the puncture and sealing the hole is done as described before.

ZUSAMMENFASSUNG

Eine verbesserte Methode für die Herstellung von dünnen Agarschichten in Polyäthylensäcken

Nach der beschriebenen Methode können schnell völlig sterile, dünne Agarschichten in Polyäthylensäcken hergestellt werden. Das Agargemisch (2,5% Agar, 0,5% Methylzellulose in Pflanzennährlösung) wird nach Sterilisation und Abkühlung bis etwa 50°C mittels Luftdruck aus dem Sterilisationsgefäss durch einen dünnen Schlauch und eine 2 mm weite Injektionskanüle in die zwischen zwei 1 mm voneinander entfernten Platten liegende inwendig sterilisierte Säcke gepresst. In der Injektionskanüle wird dem Agargemisch etwa 15 Volumprozent steriler Luft in Form von kleinen Bläschen beigemischt.

Das Agar-Methylzellulose-Gemisch nimmt im Gegensatz zu reinem Agar nach Wasserverlust schnell wieder Wasser auf. Durch darauf wachsende Pflanzen dem Nährboden entnommenes Wasser kann deshalb wiederholt ersetzt werden. Es ist notwendig, völlig sterile Keimlinge und Älchen in den Agar zu bringen. Samen können während 7 Minuten mit Sublimat 0,2%, hieraus gewachsene Keimlinge durch Benetzen mit gesättigter Ca-Hypochloritlösung und Nematoden während wenigstens 10 Minuten in 0,4% Streptomyzin-Sulphatlösung genügend desinfiziert werden. Schnittöffnungen die in den äusserlich desinfizierten Polyäthylenbeuteln angebracht werden, um Keimlinge und Nematoden hineinzubringen, werden mit einem in Äther desinfizierten Zellophanklebband verschlossen.

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Nematologica 5 (1960): 260-274. Leiden, E .J. Brill

THE MONONCHIDAE (ENOPLIDA, NEMATODA) OF NEW ZEALAND II. THE GENUS *IOTONCHUS* (COBB, 1916) ALTHERR, 1950

BY

W. C. CLARK
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COBB (1916) first erected *Iotonchus* as a subgenus of *Mononchus* Bastian, 1865 for large species with a small basal tooth or teeth, an elongated, three-ribbed stoma, a long slender tail, and single or paired reflexed ovaries and he designated *M. gymnolaimus* Cobb as the type species. ALTHERR (1950) and PENNAK (1953) first gave *Iotonchus* generic rank. Apparently unaware of this, ANDRÁSSY (1958) did the same, but placed the forms with subventral teeth in a new genus *Miconchus*. It is not possible to separate some species (e.g. *I. parazschokkei* (Allgén)) from *Mononchus* using the generic definitions of COBB (1916, 1917) or ANDRÁSSY (1958). In some species the dorsal tooth may lie partly in the anterior and partly in the posterior half of the stoma. The position of the tooth apex is not fixed and ranges over about ten per cent of the stoma length in most species. With the possible exception of *I. zschokkei* (Menzel) the stoma base is broad in *Iotonchus* and pointed in *Mononchus*. The different types of oesophago-intestinal junction in the two genera (CLARK, 1960) simplifies generic determination.

Iotonchus (Cobb, 1916) Altherr, 1950. Emend.

Mononchidae. Stoma large, barrel-shaped, with a broad base. Dorsal tooth anteriorly directed, moderate to small, often basal, but always within the posterior two thirds of stoma. Subventral teeth or denticles absent. Oesophago-intestinal junction tuberculate. Ovaries paired or single, reflexed. Testes paired opposed, outstretched. Spicules paired, free. Gubernaculum simple or with lateral processes. Lateral accessory pieces present or absent. Tail short, conoid, or long, conoid with with a cylindroid end piece or filiform. Tail in both sexes similar or different.

Found in soil, mosses, liverworts, and forest litter.

Type species *Mononchus gymnolaimus* Cobb, 1893.

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[1]) At present on study leave from Entomology Division, D.S.I.R., Nelson, New Zealand.

The known species of *Iotonchus* are:

I. acutus (Cobb, 1917) Andrássy, 1958
I. amphigonicus (Thorne, 1924) Andrássy, 1958
I. basidontus n. sp.
I. bathybius (Micoletzky, 1913) Andrássy, 1958
I. brachylaimus (Cobb, 1917) Andrássy, 1958
I. consimilis (Cobb, 1917) Andrássy, 1958
I. gymnolaimus (Cobb, 1893) Andrássy, 1958
I. jairi (Lordello, 1958) n. comb.
I. maragnus n. sp.
I. ophiocercus n. sp.
I. parazschokkei (Allgén, 1929) Andrássy, 1958
I. percivali n. sp.
I. piracicabae (Lordello, 1953) Andrássy, 1958
I. risoceiae (Carvalho, 1955) Andrássy, 1958
I. similis (Cobb, 1893) Andrássy, 1958
I. simmenensis (Kreis, 1924) Andrássy, 1958
I. tenuicaudatus (Kreis, 1924) n. comb.
I. trichurus (Cobb, 1917) Andrássy, 1958
I. zschokkei (Menzel, 1913) Andrássy, 1958

Iotonchus basidontus n. sp.
Figs. 1A-G

Ross

Holotype ♀ L = 1.81 mm; a = 30; b = 3.67; c = 6.82; V = ^{9}59.8^{7}
Allotype ♂ L = 1.95 mm; a = 30; b = 3.85; c = 8.60; T = 19.5
Paratype ♀ L = 1.68 mm; a = 32; b = 3.76; c = 7.16; V = ^{7}58.1^{8}
Awatuna-Stafford ♀ L = 1.82 mm; a = 28; b = 3.82; c = 6.85; V = ^{8}56.4^{7}
Awatuna ♀ L = 2.44 mm; a = 25; b = 4.32; c = 6.85; V = ^{11}55.2^{15}
Hongi's Track ♀♀ (n = 2) L = 1.91-1.95 mm; a = 31; b = 4.03-4.08; c = 6.45-6.66; V = $^{8-9}$58.1-59.7$^{7-8}$

Cuticle 2 μ thick, with very faint obsolescent striae. No lateral fields. Subcuticle 3 μ thick, not striated. Head narrowing anteriorly, lip region scarcely offset. Lips and papillae not prominent in lateral view. Amphid situated one half head-width behind lateral lip, aperture oval. In *en face* view lip region is hexagonal, symmetrical; lips six, equal, inner edges rounded, outer edges angular. Papillae in two rings, inner one with six, outer with ring of ten papillae. Amphid apertures crescentic in this view. In lateral view stoma is barrel-shaped, width five-eighths of the height, base broad, somewhat rounded, anterior portion not greatly in-arched. Anterior aperture half as wide as the greatest width. Dorsal tooth low, at 75 per cent of the length of the stoma, and occurring on posterior limit of anterior dorsal element. Tooth apex sharp; anterior edge of tooth concave; posterior edge straight. Apart from the presence of the tooth, dorsal and subventral anterior stoma elements similar. Postero-dorsal element thicker than subventral members, but thins towards oesophageal lumen. Suture between anterior and posterior elements clearly marked. Anterior four-fifths of stoma within a thin muscular sheath; posterior fifth embedded in locally enlarged anterior end of oesophagus. Oesophagus narrows a little to nerve ring at one-third of its length, then increases slightly to occupy half the body-width. Dorsal oesophageal gland duct at half the oesophagus length; subventral glands discharge into posterior third of oesophagus. Oesophago-intestinal junction tuberculate (Fig. 1 C). Intestine with abundant oil droplets and dark granules.

Female Vulva transverse; vagina thick walled, extending across one-third of

body. Uteri and oviducts outstretched, a sphincter muscle present between uteri and oviducts, but the glandular region, observed in other species, not clearly visible. Ovaries reflexed through one third of length of oviduct and uterus. Ovaries half as wide as body. Oocytes in a single row. Intestine passes to the left of anterior and right of posterior ovary. Prerectum absent. Rectum straight, length two-thirds anal-body width. Anal lips slightly protuberant. Tail 15 per cent of total length;

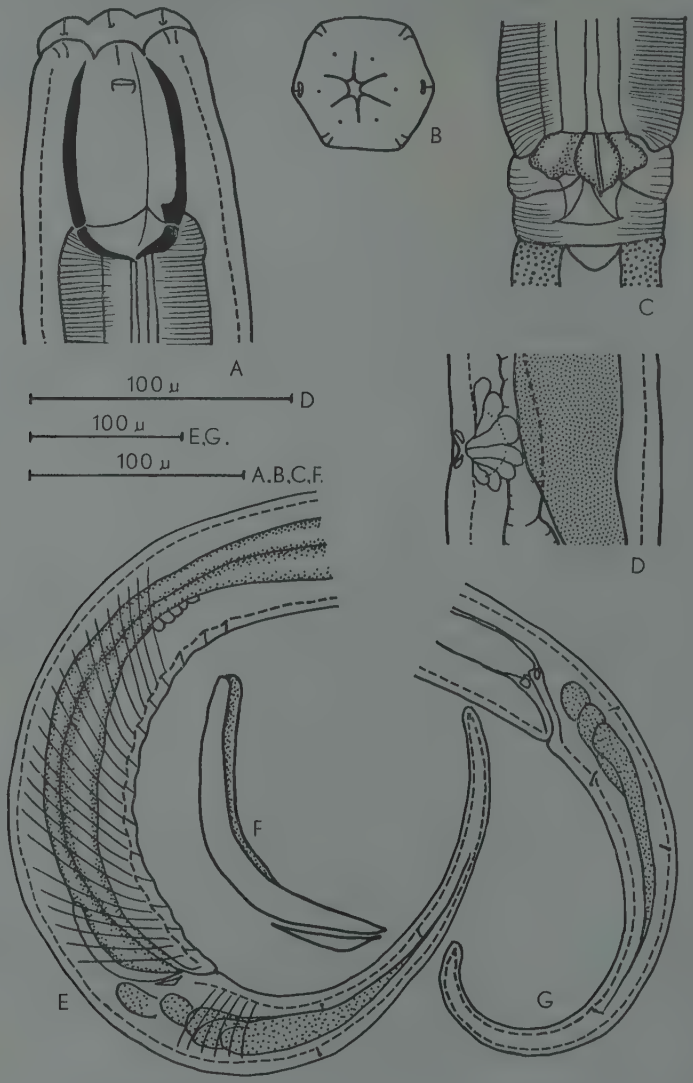

Fig. 1. *Iotonchus basidontus*. A. head region; B. *en face* view of lip region; C. oesophago-intestinal junction; D. lateral view of vulva; E. male tail; F. spicules and gubernaculum; G. female tail.

ventrally arcuate, conoid in proximal half, then cylindrical, tip rounded. Caudal glands and terminal duct present. Lateral cuticular pores as shown in fig. 1 G.

Male Testes paired, opposed, outstretched. Anterior testis five-sixths as long as posterior. Vas deferens equal to half testicular length. At a level between the second and third supplement the vas deferens swells to become the ejaculatory duct which opens into the cloaca. Prerectum equal to ejaculatory duct in length. Spicules paired, long, slender, free. Gubernaculum small, simple, without lateral processes. Lateral accessory pieces wanting. Supplements fourteen, small, papilliform, equidistant. Tail similar to female. Caudal glands with terminal duct present. Lateral cuticular pores as in fig. 1 E.

Specific differentiation. I. basidontus most closely resembles *I. gymnolaimus* (Cobb) and *I. consimilis* (Cobb); two species which need redescription. From these *I. basidontus* is distinguished by the presence of paired ovaries, the head which is not offset, the shape of the amphid (duplex in *I. consimilis*), the thickness of the stoma wall (thin in *I. gymnolaimus*) and the tail shape (tip swollen in *I. gymnolaimus*).

Type slides. In nematode collection, Entomology Division, D.S.I.R., Nelson, N.Z.

Type locality. Ross, Westland in *Lolium perenne* L.-*Trifolium repens* L.-*Anthoxanthum odoratum* L. pasture. Soil type: Hokitika fine sandy loam (a recent alluvial soil derived from schist).

Other localities. Kumara Beach, *Hydrocotyle* L. sp. -- *Trifolium repens* L. turf; Awatuna, water-logged soil with *Ranunculus* L., *Hydrocotyle* L. and *Juncus* L.; Awatuna-Stafford, *Carpodetus serratus* Forst.-*Weinmannia racemosa* Linn. fil. scrub; Hokitika, mental hospital terrace, swampy land with sedges, *Juncus* L., and *Drosera* L. spp.; Hongi's Track, *Beilschmiedia Tawa* Benth. & Hook f.-*Knightia excelsa* R. Br. forest litter; Kiripaka, *Beilschmiedia Taraire* Benth. & Hook. f.-*Vitex lucens* T. Kirk forest litter.

Remarks Several specimens had rhabditids and tylenchids in the intestine. There appear to be no grounds for separating the Awatuna female from the others, though the measurements indicate a larger animal with a slightly shorter oesophagus.

Iotonchus maragnus n. sp.

Figs. 2 A-I

Sergeants Hill
Holotype ♀ L = 4.22 mm; a = 69; b = 7.14; c = 2.95; V = ^{6}35.9^{7}
Paratype 1 ♀ L = 3.64 mm; a = 59; b = 6.33; c = 2.31; V = ^{15}38.9^{10}
Westport Pakihi
Allotype ♂ L = 3.3 mm; a = 53; b = 6.24; c = 2.66; T = 24

Cuticle 1.5 μ thick with obsolescent fine annuli in caudal region. No lateral fields. Subcuticle 2.5 μ thick. Body tapers slightly anteriorly; lip region offset by slight constriction. In lateral view lips closely approximated. Amphids small, cup-

like, aperture a transverse slit. In *en face* view lip region almost circular; divided into six equal segments. Inner lip margins pointed, outer margins rounded. Papillae in two rings; inner ring of six and outer ring of ten (two papillae on each

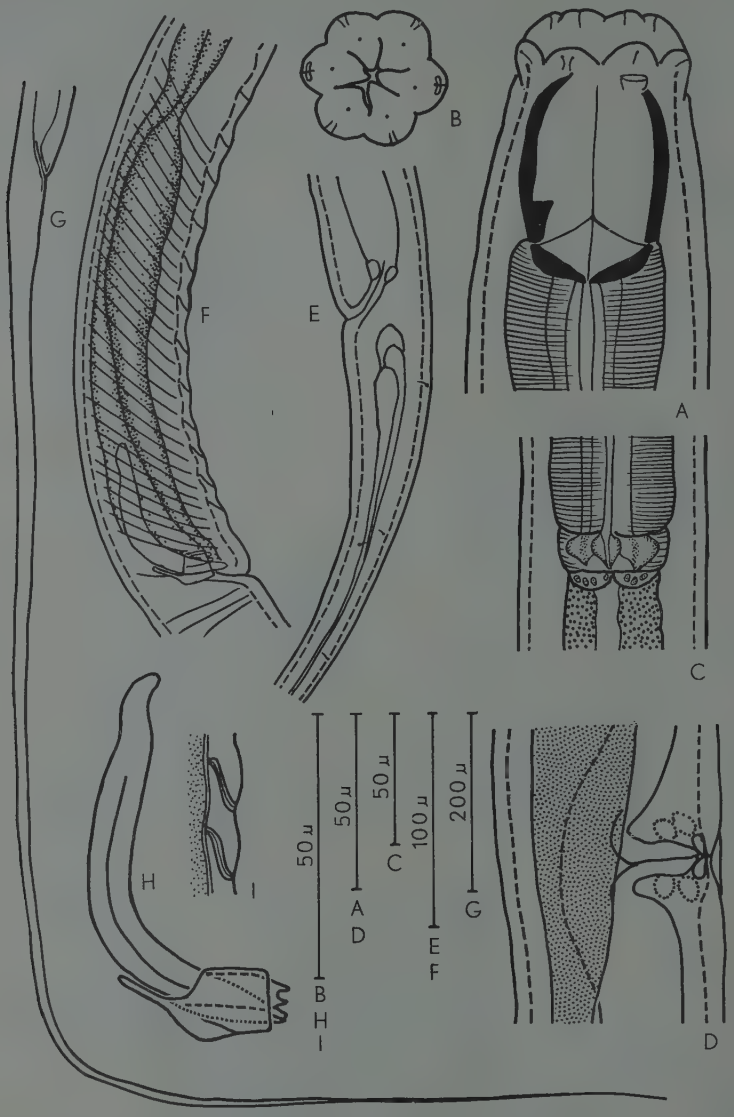

Fig. 2. *Iotonchus maragnus*. A. head region; B. *en face* view of lip region; C. oesophago-intestinal junction; D. lateral view of vulva; E. female tail; F. anal region of male; G. female tail; H. spicule, gubernaculum and lateral accessory piece; I. supplements.

of the submedian lips and one on each lateral). Amphid apertures crescentic in this view.

In lateral view stoma barrel-shaped, broad at base, three-quarters as wide as long, length equal to head width. Dorsal tooth apex at 59-61 per cent of stoma length. Anterior edge of tooth straight, horizontal; posterior edge intersects dorsal stoma wall at suture between anterior and posterior elements. Apart from dorsal tooth, ventral and dorsal stoma walls similar, in-arched anteriorly. Anterior stoma aperture two-fifths of greatest stoma width. Posterior elements similar. Outer surface of stoma with fine transverse striae. Anterior four-fifths of stoma within thin muscle sheath; posterior fifth embedded in expanded end of oesophagus.

Oesophagus narrows a little to nerve ring at one-third of its length where it occupies half body-width, then gradually expands to three-fifths body-width. Oesophageal gland ducts occur at 44, 63-67, and 93-96 per cent of the oesophageal length. Oesophago-intestinal junction tuberculate, funnel-shaped valve not seen. Posterior face of sphincter muscle with several large glandular (?) cells. Intestine as usual.

Female Vulva transverse. Vagina thick walled, extending across one third to half body-width. No radiating muscles seen. Uteri paired, opposed, outstretched, with conspicuous sphincter muscle and glandular region at uterus-oviduct junction. Ovary half as wide as body, reflexed through one sixth length of oviduct and uterus. No pre-rectum. Rectum straight, equal to anal-body-width. Tail extremely attenuated, filiform, approximately one-third of body length. Caudal glands present, terminal duct absent. Caudal cuticular pores as in fig. 2 E.

Male Testes paired, opposed, outstretched. Length of testes equal to length of vas deferens. Vas deferens enlarged posterior to second supplement where it becomes the ejaculatory duct. Ejaculatory duct and prerectum equal. Supplements twelve, small, low, almost equidistant. Spicules paired, free, slender, curved, with thickened median strand in distal three-quarters, tips bifid, head sigmoid. Paired, notched, lateral accessory pieces present. Gubernaculum large, thickened behind the spicules, lateral processes large, thin, partly enveloping the spicules and lateral accessory pieces. Tail similar to female.

Specific differentiation. The extremely long whip-like tail 34-43 per cent of the total length readily separates *I. maragnus* from all other species in the genus except *I. trichurus* Cobb and *I. percivali* n. sp. It is distinguished from *I. trichurus* by the paired ovaries and larger size and from *I. percivali* by the more anterior position of the vulva, the slightly longer tail and the smaller size. In the only males available there are twelve supplements in *I. maragnus* and eighteen in *I. percivali.*

Type slides. Entomology Division, D.I.S.R., Nelson, N.Z.

Type locality. Sergeant's Hill, Pakihi, N.Z.M.S.1. S23/24, 135724, 30 metres. Vegetation *Gleichenia circinata* Swartz, *Leptospermum scoparium* Forst., *Cladium teretifolium* R. Br., *Sphagnum falcatulum* Bresch. Soil: Sergeant series (gley podsols).

Other locality. Westport Pakihi, vegetation as above.

Remarks A nativorous species; food in the intestine includes larvae of its own species. Both localities with wet, mature gley podsol soils. The specific name alludes the whip-like tail (Greek μάραγνα = whip).

Iotonchus ophiocercus n. sp.
Figs. 3 A-H

Holotype ♀ (Barrytown) L = 4.03 mm; a = 41; b = 4.75; c = 4.88; V = ^{12} 56.6 ^{10}

Allotype ♂ (Waimangaroa) L = 3.82 mm; a = 46; b = 5.17; c = 5.76; T = 15

Paratypes (Barrytown) 2 ♀♀ L = 3.82 mm; a = 45-47; b = 4.81-4.85; c = 4.68; V = $^{9-10}$ 53.9-61.9 $^{5-10}$

(Kumara Beach) ♀ L = 3.98; a = 44; b = 4.92; c = 5.21; V = 54.5

♂ L = 3.75 mm; a = 38; b = 4.58; c = 10.5; T = 24.6

Cuticle 3 μ thick, with exceedingly fine annuli only visible under very favourable conditions, usually appearing smooth. No lateral fields. Subcuticle 2 μ thick. Lip region scarcely offset. Lips rather closely approximated. Amphids cup-shaped; aperture narrow, elliptical, situated at level of anterior limit of stoma walls. *En face* view (of larva) shows a rounded hexagonal lip region, lips equal, inner edges pointed. Papillae in two rings, inner ring of eighteen papillae, (three on each lip arranged in an obtuse triangle), outer ring of ten papillae. Abutting edges of the stomal elements slightly thickened on the inner side only. In lateral view stoma barrel-shaped, broadly rounded at base; anterior aperture four-fifths of maximum stoma width. Dorsal tooth apex at 54-60 per cent of stoma length. Tooth apex sharply pointed, anterior edge slightly convex, almost horizontal; lower edge slightly convex in middle and merging with lower stoma wall a short distance anterior to the suture between the anterior and posterior elements. Dorsal wall indented below tooth. Anterior parts of dorsal and ventral walls similar, incurved anteriorly, ventral wall appears slightly thicker due to thickening at abutting edges of subventral elements. Dorsal posterior element shorter and thicker than subventrals. Suture between anterior and posterior elements clearly marked. Outer surface of stoma with fine transverse striae. Anterior four-fifths of stoma enveloped in thin muscle sheath; posterior fifth embedded in locally expanded oesophagus.

Oesophagus narrows rapidly behind stoma base to about half body-width and remains this width to nerve ring at one-third of its length, then gradually expanding to half body-width in conformity with general body contour. Dorsal oesophageal gland opens into oesophagus near middle; subventral glands discharge into posterior third.

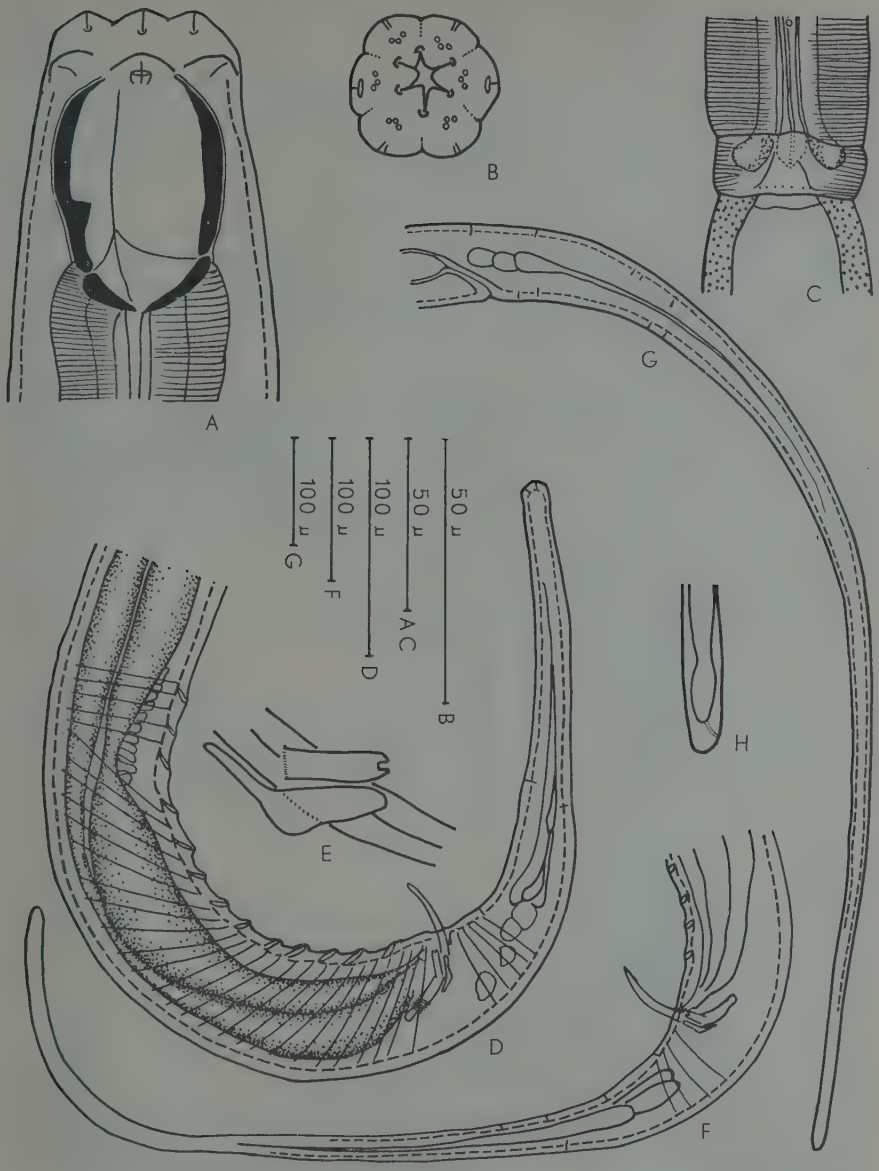

Fig. 3. *Iotonchus ophiocercus*. A. head region; B. *en face* view of lip region of larva; C. oesophago-intestinal junction; D. tail of paratype male; E. detail of gubernaculum, lateral accessory piece and spicule; F. tail of male allotype; G. female tail; H. tail tip.

Oesophago-intestinal junction tuberculate. Intestine a simple tube eight to twelve cells in diameter; cells packed with oil globules and dark granules.

Female Vulva transverse. Vagina with well developed musculature, extending half way across body. Uteri paired, opposed, outstretched, with a well developed sphincter muscle and glandular zone at the uterus-oviduct junction. Ovary reflexed through one-fifth of oviduct and uterus length. Ovary half as wide as body. A few cuticular pores sometimes present in the region of the vulva. Intestine passes to right of anterior ovary and to left of posterior one. Prerectum absent. Rectum straight, one anal-body-width long. Posterior anal lip slightly protuberant.

Tail very long, equal to one-fifth of the body length; tapering smoothly, ventrally arcuate, tip rounded. Caudal glands and sub-terminal duct present. Cuticular pores as shown in figure 3 G.

Male Testes paired, outstretched, opposed, sperms abundant; not arranged in any pattern. Vas deferens equal to testicular length; greatly dilated posterior to third supplement to become ejaculatory duct. Anterior half of ejaculatory duct much wider than posterior. Prerectum commences opposite fourth supplement. Spicules paired, free, slender, especially in distal half; heads knobbed; one and a quarter anal-body-widths long. Gubernaculum similar to that of *I. maragnus*. Lateral accessory pieces notched. Supplements eleven or thirteen, each with a low rounded papilla. Allotype male tail similar to female. Paratype male tail much shorter than other male or females. Caudal glands present; terminal duct not seen.

Specific differentiation. I. ophiocercus is unique in possessing a long tail (c = about 5), and a tooth the apex of which lies near the middle of the stoma. Some species have a tail as long, or longer than *ophiocercus* (e.g. *trichurus, tenuidentatus, maragnus* and *percivali),* but all these have the tooth apex clearly in the posterior half of the stoma.

Type slides. All in Nematode Collection, Entomology Division, D.S.I.R., Nelson, N.Z.

Type locality. Johnson's Reserve, Barrytown, N.Z.M.S.1., S 37, 847223, 30 metres, in forest dominated by *Weinmannia racemosa* Linn. f. and tree ferns. Soil Ikamatua fine sandy loam (an immature podsol).

Other localities. Kumara Beach in *Hydrotyle* L.-*Trifolium repens* L. turf; Craigielachie, Otago in *Agrostis tenuis* L.-*Dactylis glomerata* L.-*Trifolium repens* L. pasture; Collingwood Pakihi, *Cladium teretifolium* R. Br., *Gleichenia circinata* Swartz, *Drosera pygmaea* DC.; Wharekohe, *Gleichenia circinata* Swartz, *Juncus* L. sp. *Leptospermum scoparium* Forst.

Remarks. This species has been collected from wet podsolic soils only.

Many specimens had nematode remains in the intestine; dorylaimoid spears and spicules as well as *I. ophiocercus* stomas have been seen.

The name is derived from the nature of the tail (ὄφις — a serpent, κέρκος — a tail).

Iotonchus parazschokkei (Allgén)

Figs. 4 A-F

Mononchus parazschokkei Allgén, 1929 p. 224 figs. 6a, b.
Mononchus (*Iotonchus*) *parazschokkei* Goodey 1951 p. 272
Iotonchus parazschokkei Andrássy 1958 p. 159

Males unknown. (For measurements of females see Table I).

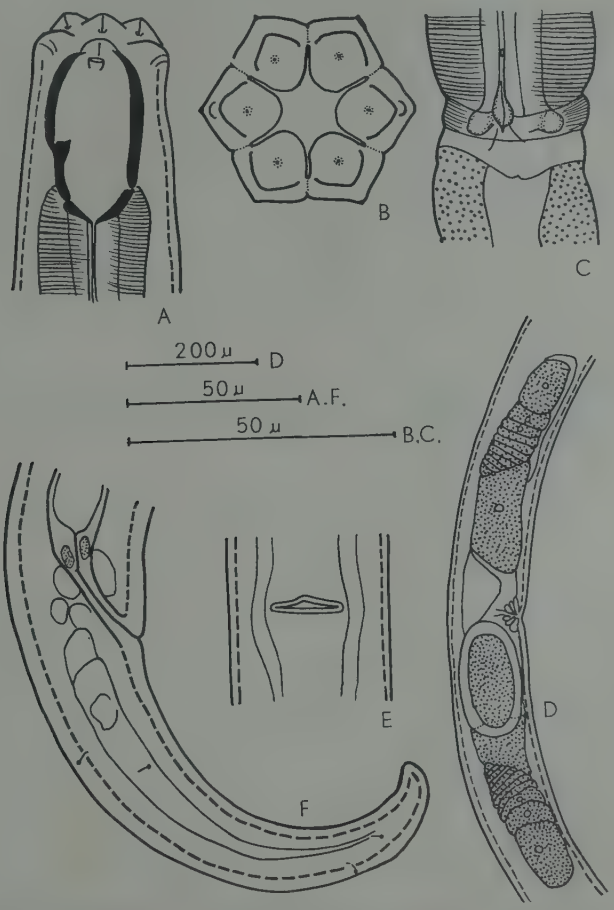

Fig. 4. *Iotonchus parazschokkei*. A. head region; B. *en face* view of lip region; C. oesophago-intestinal junction; D. female reproductive organs; E. ventral view of vulva; F. female tail.

Cuticle smooth, 3 μ thick; no lateral fields. Subcuticle 1 μ thick. Lip region scarcely offset. Amphids small, cup-like; aperture oval, situated posterior to outer lateral cephalic papilla. In *en face* view lip region hexagonal; lips equal and symmetrical, inner lips rounded, outer lips angular; inner ring with six papillae, outer

TABLE I

Measurements of Iotonchus parazschokkei *females*

	Kerikeri	*Rerewhakaitu*	*Hongi's Track*	*Mt. Messenger*	*Arthur's Pass*	*Barrytown*	*Waitaanga Saddle*	*Lake Mahinapua*
n	4	10	2	1	18	2	10	11
L (mm) mean	1.28	1.50	1.57	1.79	1.90	2.20	2.34	2.37
S.D. (mm)	± 0.041	± 0.111	–	–	± 0.151	–	± 0.207	± 0.183
Range	1.21–1.31	1.27–1.61	1.49–1.65	1.79	1.67–2.11	2.13–2.26	1.84–2.57	2.04–2.64
a	25–35	27–34	27–28	36	27–34	28–29	29–35	29–37
b	3.4–3.5	3.7–4.2	3.6	3.9	3.7–4.2	4.0–4.1	3.7–4.6	3.8–4.6
c	10.7–11.2	10.9–14.2	9.5–9.8	8.4	10.9–14.2	12.8–14.9	9.3–15.2	8.8–13.4
a.o. [1])	8–11	6–17	8–9	12	6–17	11–14	10–18	6–14
V	61–63	58–65	61–63	59.3	58–65	62–63	58–63	57–61
p.o. [1])	8–10	8–13	8–10	9	8–13	11–14	10–16	8–13

[1]) a.o. and p.o. anterior and posterior ovaries respectively.

ring with ten. Inner papillae situated on eminences near middle of each lip; outer papillae near the tips of the lips. Stoma circular in cross section.

In lateral view stoma heavily sclerotized, ovate, anterior end widest. Stoma widest at 47-55 per cent of its length. Dorsal tooth apex occurs at 46-56 per cent of the stoma length. Anterior edge of tooth straight or nearly so, sloping slightly posteriorly. Posterior edge of tooth slightly convex, quickly merging with general contour of posterior portion of stoma wall. Posterior to dorsal tooth outer surface of stoma is indented. Anterior to tooth dorsal wall is similar to, but thinner than the gently curving, anterior, ventral wall. The dorsal posterior element thicker and shorter than ventral ones. Posterior fifth of stoma embedded in slightly expanded end of oesophagus. Anterior four-fifths surrounded by a thin muscle sheath.

Oesophagus narrows a little to nerve ring, at one-third of its length, and then expands again, generally occupying half of body width. Oesophago-intestinal junction tuberculate with a well developed sphincter muscle. Intestine as usual. Vulva a transverse slit, one-third as wide as body, with thickened lips. Vagina thick walled, extending half way across body. Uteri paired, outstretched, opposed, and reflexed through half length of oviduct and uterus. Usually one or two eggs in the uteri at a time.

No prerectum. Rectum slightly arcuate, one to one and a quarter anal-body-widths long. Tail short, conoid, ventrally arcuate. Caudal glands present. No terminal duct. Three pairs of lateral cuticular pores present distributed as in fig. 4 F.

Specific differentiation. I. parazschokkei is similar to *I. amphigonicus* (Thorne) and *I. zschokkei* (Menzel), but is distinguished by the longer tail.

Localities. Arthur's Pass and Takaka Hill in *Nothofagus* Blume forest litter; Lake Mahinapua in *Dacrydium cupressinum* Soland-*Quintinia acutifolia* T. Kirk forest litter; Barrytown in *Weinmannia racemosa* Linn. f.-tree fern litter; Four Mile River, Buller, and Sergeant's Hill from soil under *Gleichenia circinata* Swartz and *Sphagnum*; Iron Hill, Cobb River soil under *Carpha alpina* R. Br.; Kerikeri soil under *Leptospermum scoparium* Forst. and *Hakea acicularis* R. Br.; Kiripaka *Beilschmiedia Taraire* Benth. & Hook. f.-*Vitex lucens* T. Kirk forest litter; Hongi's Track *Beilschmiedia Tawa* Benth. & Hook. f.-*Knightia excelsa* R. Br. forest litter; Rerewhakaitu soil under *Leptospermum scoparium* Forst. and *Poa caespitosa* Forst.; Waiotapu *Dacrydium cupressinum* Soland.-*Weinmannia racemosa* Linn. f. forest litter; Waitaanga Saddle in liverworts and mosses; Mt. Messenger *Olea Cunninghamii* Hook. f.-*Vitex lucens* T. Kirk forest litter.

Remarks. This species varies in length, but there are no conspicuous breaks in the series of measurements, or morphological differences between the largest and smallest specimens. It inhabits soil, moss and litter.

Iotonchus percivali n. sp.

Figs. 5 A-I

Holotype ♀ (Runanga) L = 5.94 mm; a = 71; b = 5.02; c = 3.79; V = ^{13}51.5^{13}

Allotype ♂ (Reefton) L = 6.10; a = 74; b = 5.06; c = 4.15; T = 31

Cuticle 4 μ thick, smooth on body, annulated on tail. No lateral fields. Subcuticle 3 μ thick. Lip regions slightly offset, lips closely apposed, almost fused. Amphids cup-like; aperture narrow, situated near anterior end of sclerotized stoma. In *en face* view lip region circular, lateral lips smaller and more pointed on inner edge than sub-median lips. Outer edge of lips rounded. Papillae in two rings of six each. In lateral view sclerotized stoma is barrel-shaped, base broad; greatest width at one-quarter of its length where it occupies three-quarters of body-width. Dorsal tooth apex at 63 per cent of stoma length. Anterior edge of tooth straight, horizontal; apex rather blunt. Posterior edge of tooth intersects stoma wall at suture between anterior and posterior elements. Dorsal and ventral walls similar. Dorsal posterior element slightly thicker than subventrals. Suture between anterior and posterior elements distinct. Outer surface of stoma transversely striated. Subdorsal and sub-ventral cuticular pores near widest part of stoma. Muscular sheath about anterior part of stoma very thin. Anterior end of oesophagus expanded about stoma base, then narrowing to half body-width at nerve ring at one-fifth of oesophageal length, gently expanding beyond this to two-thirds body-width. Oesophago-intestinal junction tuberculate. Intestine with abundant oil droplets and dark granules.

Female Vulva transverse. Vagina thick walled, extending across half body-width. Uteri paired, outstretched, opposed, a strong sphincter and glandular region at uterus-oviduct junction. Upper part of uterus in holotype filled with sperm. Ovary half as wide as body, reflexed through one-third length of oviduct and uterus. Intestine passes to left of anterior ovary, and to right of posterior one. No prerectum; rectum slightly arcuate, length almost equal to anal-body-width. Tail ventrally flexed when fixed, long (26 per cent of body length), filiform, tip rounded. Caudal glands and terminal duct present. Cuticular pores few on proximal part of tail, more common on annulated distal two thirds.

Male Testes paired, opposed, outstretched, with abundant sperm randomly arranged. Vas deferens length equal to both testes. Opposite first supplement vas deferens expands to become ejaculatory duct, and the intestine narrows to form prerectum. Spicules paired, free, moderately heavy, knobbed on upper end with a thickened median rib on distal 85 per cent. Gubernaculum with thickened keel behind spicules and with lateral pieces which flank spicules. Parallel to and a little in front of lateral processes are notched, lateral accessory pieces. Supplements eighteen, low, papilliform, with rather sigmoid "cores". Tail similar to female.

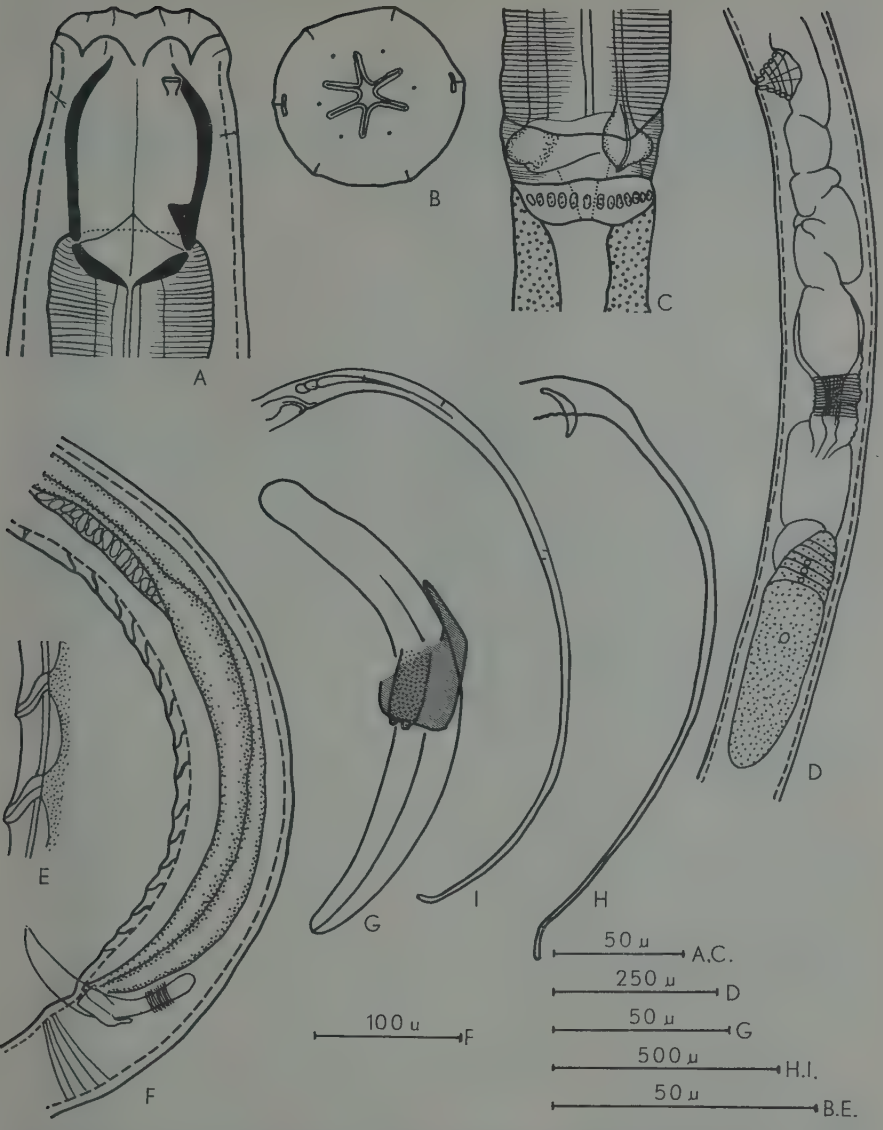

Fig. 5. *Iotonchus percivali*. A. head region; B. *en face* view of lip region; C. oesophago-intestinal junction; D. posterior limb of female reproductive system; E. male supplements; F. anal region of male; G. spicule, gubernaculum and lateral accessory piece; H. male tail; I. female tail.

Specific differentiation. Another species of the long-tailed group of *Iotonchus;* readily distinguished from all others by its large size, paired ovaries, median vulva and long tail.

Type slides. Entomology Division, D.S.I.R., Nelson, N.Z.

Type locality. Runanga, Westland, N.Z.M.S.1. S44, 774951, 20 metres. Very wet pacture, *Holcus lanatus* L., *Agrostis tenuis* L., *Juncus* L. sp., *Ranunculus repens* L.; soil: Karangarua fine sandy loam—a recent, poorly drained alluvial soil.

Other locality. Reefton in *Sphagnum* swamp.

Remarks. This species is named in honour of my teacher, the late Prof. E. Percival, of the University of Canterbury.

ZUSAMMENFASSUNG

Die Mononchiden (Enoplida, Nematoda) Neu-Seelands. 2. Die Gattung Iotonchus *(Cobb, 1916) Altherr 1950.*

Iotonchus (Cobb, 1916) Altherr 1950 wurde neu definiert. Die Gattung unterscheidet sich von *Mononchus* Bastian durch die tuberkulate Verbindung vom Oesophagus zum Darm, den Dorsalzahn, der ganz oder hauptsächlich in der hinteren Hälfte der Mundhöhle liegt, und durch die breite Basis der Mundhöhle. *I. jairi* (Lordello, 1959) n. comb. und *I. tenuidentatus* (Kreis 1924) n. comb. wurden aus der Gattung *Mononchus* in die Gattung *Iotonchus* überführt.

I. basidontus n. sp. unterscheidet sich von *I. gymnolaimus* und *I. consimilis* durch den Besitz von paarigen Ovarien. *I. maragnus* n. sp. ist durch den Besitz eines sehr langen fadenförmigen Schwanzes (c = 2,3 bis 2,9) und die Lage der Vulva (V = 36-39) charakterisiert. *I. ophiocercus* n. sp. ist die einzige langgeschwänzte Art der Gattung mit der Zahnspitze nahe der Mundhöhlenmitte. *I. percivali* n. sp. ist die grösste Art der Gattung (L = 6 mm). Sie hat einen langen Schwanz (c = 3,8 bis 4,1), eine mittelständige Vulva und paarige Ovarien. *I. parazschokkei* (Allgén 1929) wurde neubeschrieben.

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THE MONONCHIDAE (ENOPLIDA, NEMATODA) OF NEW ZEALAND III. A REVIEW OF THE GENUS *COBBONCHUS* ANDRÁSSY, 1958 WITH DESCRIPTIONS OF NEW SPECIES

BY

W. C. CLARK

Rothamsted Experimental Station, Harpenden, Herts., England [1])

ANDRÁSSY (1958) erected the genus *Cobbonchus* for three species, *M. palustris* Cobb, *M. radiatus* Cobb and *M. teres* Cobb, which COBB (1917) had referred to *Mononchus* Bastian. *Cobbonchus* is characterised by the possession of an anterior dorsal tooth and two posterior subventral teeth.

None of these species was fully described. *C. radiatus* and *C. teres* are based on "a single young specimen" and a "single more or less shrunken specimen" respectively. Cobb did not remark on the rounded head of *C. palustris* which is quite unlike that of any other mononchid.

Miss E. M. Buhrer (*in litt.*) considers it unlikely that the holotypes of these species still exist. When Cobb based a species on a single specimen it was usually beheaded or sectioned. Many were mounted in glycerine jelly which later proved unsatisfactory. However, Miss Buhrer has supplied photographs of Cobb's original drawings, including unpublished drawings of the tails which are reproduced here in figs. 1-3. From these figures and Cobb's description I have compiled the fuller descriptions given below.

Since Andrássy's work appeared, more information has become available about the genus *Cobbonchus,* which is redefined below.

Cobbonchus Andrássy, 1958 Emend.

Mononchidae. Lip region scarcely or not offset. Stoma moderately large, about twice as long as broad, with three anteriorly directed teeth. Dorsal tooth larger than, and anterior to subventral teeth. Amphids small to moderate, transverse, slit like. Ovaries paired or single. When single either the anterior or posterior ovary may be suppressed and represented by a uterine sac [2]). Vulva post-median, vagina often muscular. Oesophago-intestinal junction non-tuberculate (CLARK, 1960). Testes paired, opposed, outstretched. Spicules simple, paired, free. Gubernaculum complex. Supplements simple, papilliform, equidistant. Tail in both sexes short,

[1]) At present on study leave from Entomology Division, D.S.I.R., Nelson, New Zealand.

[2]) I have a single female of a new species from Stafford, N.Z., which has a posterior ovary and a pre-vulval uterine sac. As this is a most unusual feature, I await more material before describing it.

ventrally arcuate, conoid at first, becoming cylindroid distally. Caudal glands massive, often reaching beyond the anus; ampulla well developed, terminal duct in all known species. Cuticular pores not known in females, on papillae in only known males (*C. pounamua* n. sp.).

Found in wet soil or *Sphagnum* bogs.

Type species (by author's designation): *Mononchus palustris* Cobb, 1917.

The known species of the genus are:

C. chauliodus n. sp.
C. mauritianus (Williams, 1958) Clark, 1960
C. palustris (Cobb, 1917) Andrássy, 1958
C. pounamua n. sp.
C. radiatus (Cobb, 1917) Andrássy, 1958
C. teres (Cobb, 1917) Andrássy, 1958

Cobbonchus palustris (Cobb) Andrássy

Figs. 1 A-C

Mononchus (*Mononchus*) *palustris* Cobb, 1917, p. 154, fig. 18.
Mononchus palustris Schneider, 1937, p. 49, fig. 6a-b.
Cobbonchus palustris Andrássy, 1958, p. 161

Female (after Cobb) L 1.4 mm; a 37; b 3.33; c 33.3; V 62.

Lip region not offset, head rounded. Papillae in two rings of six each. Amphid aperture small, transverse, at level of dorsal tooth apex. Stoma large, almost twice as long as broad, parallel-sided. Stoma wall indented in region of teeth, base broad. Dorsal tooth apex at 32 per cent of stoma length; subventral tooth apices at 57 per

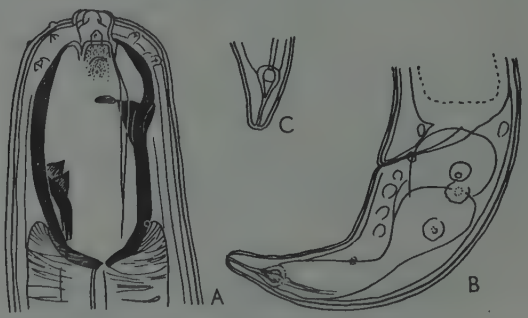

Fig. 1. *Cobbonchus palustris*. A. lateral view of head; B. female tail; C. tail tip. (All after Cobb).

cent, posterior fifth of stoma embedded in anterior end of oesophagus. Nerve ring at 8 per cent of body length. Intestine 12-15 cells in girth. Ovaries paired, rather small, tapering, eight to ten oocytes in each. Eggs elongated, twice as long as body width. Rectum straight, length equal to 0.7 of anal-body-width. Tail short, ventrally arcuate, rather conoid. Caudal glands broad, saccate; ampulla large, duct terminal.

Recorded by Cobb from a white cedar swamp, Jefferson County, Wisconsin, U.S.A.

Cobbonchus radiatus (Cobb) Andrássy
Figs. 2 A-B

Mononchus (*Mononchus*) *radiatus* Cobb, 1917, p. 154, fig. 17.
Cobbonchus radiatus Andrássy, 1958, p. 161.

"Young specimen" (after Cobb) L 0.8 mm; a 35; b 3.33; c 20; V 74

Lip region offset by a broad shallow constriction. Papillae in two rings, an inner one of six and an outer one of ten. Amphid aperture a small crescentic slit at level of dorsal tooth apex. Stoma large, almost twice as long as broad (figured specimen appears a little distorted). Dorsal tooth apex at 24 per cent of stoma length; subventral tooth apices at 64 per cent. Cobb's figures show two unusual structures posterior to the stoma base which I am unable to explain. Nerve ring at 9 per cent of body length. Genital organs apparently paired and symmetrically

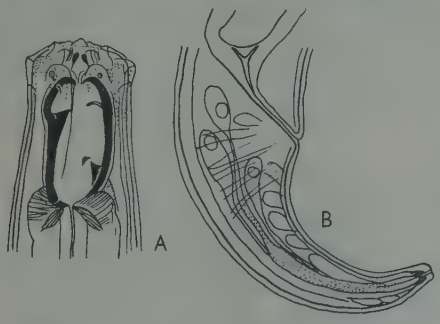

Fig. 2. *Cobbonchus radiatus*. A. lateral view of head; B. female tail. (Both after Cobb).

reflexed. Rectum almost straight, nearly equal to anal-body-width. Anal lips slightly protuberant. Tail short, ventrally arcuate, conoid. Caudal glands rather slender for the genus, ampulla present, duct terminal.

Specimen from "patch of wild cranberries with sphagnum", Cranberry Bog, New Lisbon, New Jersey, U.S.A.

Remarks. Cobb's sketches include an outline from which the measurements were taken. This shows the anterior and posterior limits of the reproductive system, indicating that the specimen may have been a young adult female.

Cobbonchus teres (Cobb) Andrássy
Figs. 3 A-B

Mononchus (*Mononchus*) *teres* Cobb, 1917, p. 155, fig. 19.
Cobbonchus teres Andrássy, 1958, p. 161.

"Young specimen" (after Cobb) L 0.6 mm; a 26; b 2.86; c 16.5; V 60

Lip region not offset, head squarish. Papillae in two rings of six each. Amphid apertures small crescentic slits at level of dorsal tooth apex. Stoma large, broad, almost barrel-shaped. Dorsal tooth apex at 27 per cent of stoma length; subventral tooth apices at 65 per cent. Posterior fifth of stoma embedded in anterior end of oesophagus. Nerve ring at 8 per cent of body length. Rectum almost sigmoid,

length a little less than anal-body-width. Anal lips protuberant. Tail strongly arcuate, conoid. Caudal glands elongated, ampulla conspicuous, one fifth as long as tail, duct terminal.

Remarks. Cobb noted "description prepared from a single, more or less shrunken specimen found in the sphagnum of a pot in which blueberry plants were being cultivated".

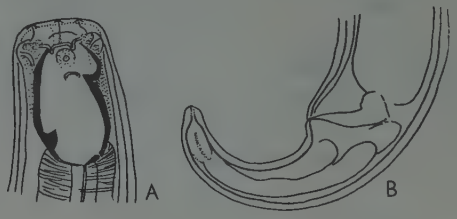

Fig. 3. *Cobbonchus teres.* A. lateral view of head; B. female tail. (Both after Cobb).

Cobbonchus mauritianus (Williams) Clark
Figs. 4 A-C

Mononchus mauritianus Williams, 1958, p. 5-6, figs 5 & 6.
Cobbonchus mauritianus Clark, 1960, p. 182.

Measurements ♀♀ (after Wiliams) L 1.1-1.3 mm; a 32; b 3.16; c 24.4; V 63.2

Cuticle 1 μ thick, smooth, no lateral fields; subcuticle 1 μ thick. Lip region truncate, slightly offset. Body tapering gradually towards head. Papillae in two rings, inner one of six, outer of ten papillae. Amphid aperture of moderate size,

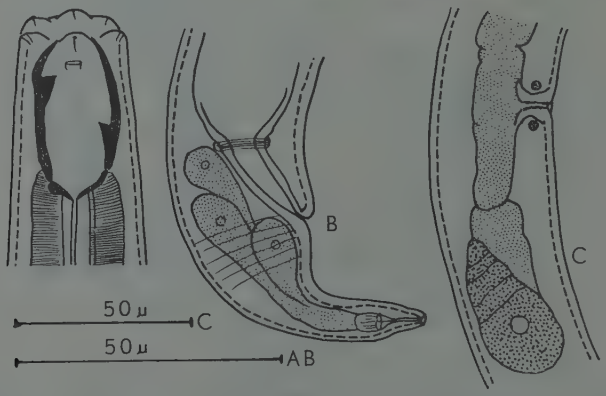

Fig. 4. *Cobbonchus mauritianus.* A. lateral view of head; B. female tail; C. posterior branch of female reproductive system.

a transverse slit situated a little anterior to dorsal tooth apex. Stoma parallel-sided, large, almost twice as long as wide. Dorsal tooth apex 23-24 per cent of stoma length; subventral tooth apices at 53-57 per cent. Posterior sixth of stoma embedded in anterior end of oesophagus. Oesophagus narrowing a little from stoma base to nerve ring at 23 per cent of its length, then expanding gently to three-

quarters body-width at the widest part. Oesophageal gland ducts occur at 39, 73 and 94 per cent of oesophageal length. Oesophago-intestinal junction non-tuberculate. Conical structure on posterior face of sphincter muscle prominent in my specimen. Vagina a thick walled tube with a sphincter muscle, but without radiating muscle fibres. Uteri and oviducts paired, outstretched; ovaries short reflexed through half length of oviduct and uterus. No prerectum. Rectum straight, almost equal to anal-body-width. Anal lips prominent. Tail short, ventrally flexed, conoid at first, then cylindroid. Caudal glands and ampulla large, prominent. Terminal duct slightly dorsal. No cuticular pores seen.

Remarks. The above description was prepared from a single paratype female kindly supplied by Mr. J. R. Williams. *C. mauritianus* differs from *C. palustris* (Cobb) in the shape of the head, the position of the teeth, and the bulbous anal region, but not in the shape of the tail as suggested by WILLIAMS (1958). Williams has informed me (*in litt.*) that the soil on Plaine Sophie, Mauritius where these specimens were collected is very wet, indicating that this species, like all others in the genus, has a preference for wet situations.

Cobbonchus chauliodus n. sp.
Figs. 5 A-D

Holotype ♀ L 3.28 mm; a 51; b 3.12; c 40.6; V 19 69.9

Cuticle 1.5 μ thick, smooth except on cylindroid part of tail where it is annulated, no lateral fields. Subcuticle 2 μ thick. Lip region slightly offset. Papillae in two rings; inner ring of six and outer ring of ten. Amphids cup-like, situated a little behind dorsal tooth apex; aperture slitlike. Stoma heavily sclerotized, widest anteriorly, narrowing towards the base. At widest part stoma occupies 70 per cent of body width. Walls inarched anteriorly. Dorsal posterior stoma element appears pyriform in optical section; subventrals appear lenticular, all elements thinning towards oesophageal lumen. Outer surface of stoma with fine transverse striae. Single dorsal tooth massive, apex at 16 per cent of stoma length and reaching almost to centre of stoma, anterior edge straight sloping posteriorly from apex, posterior edge merging with stoma wall near midpoint of stoma. Subventral teeth much smaller than dorsal tooth, apices at 39 per cent of stoma length, anterior edges concave, sloping posteriorly, posterior edges convex. Anterior four-fifths of stoma surrounded by a thin sheath, posterior fifth embedded in anterior end of oesophagus. Oesophagus of uniform diameter from stoma base to nerve ring at one sixth of its length, then expanding a little to occupy three-fifths of body-width. Oesophageal gland ducts occur at 45, 67, and 96 per cent of oesophageal length. Oesophago-intestinal junction non-tuberculate, details obscure, but appears essentially the same as *Prionchulus muscorum* (Dujardin). Intestine passes to left of reproductive organs. Vulva transverse. Vagina extending across one third of body width, broad. Vulva and vagina surrounded by massive musculature. Uterus prodelphic, doubly reflexed in distal part in holotype (fig. 5), posterior uterus represented by a long post-vulval sac. Sphincter muscle present at uterus-oviduct

junction. Ovary rather small, reflexed less than one fifth of the length of oviduct and uterus. Rectum straight, length equal to anal-body-width. Anterior anal lip lobar in form. Tail short, conoid at first with a ventral flexure, after which it is cylindroid with a terminal peg. Caudal glands very large occupying most of the post-anal space. Ampulla well developed, duct terminal. One cuticular pore near end of cylindroid part of tail.

Specific differentiation. The large size immediately separates *C. chauliodus* from all other species in the genus. Other distinguishing features are the massive dorsal tooth, and the single prodelphic ovary.

Type slides Holotype and paratype (larva) in nematode collection, Entomology Division, D.S.I.R., Nelson, N.Z.

Type locality. Collingwood Pakihi, Nelson, N.Z.M.S.1. S3, 086013, 30 metres, in a gley podsol soil with *Cladium teretifolium* R. Br., *Gleichenia circinata* Swartz, *Drosera pygmaea* DC. and *Celmisia gracilenta* Hook f.

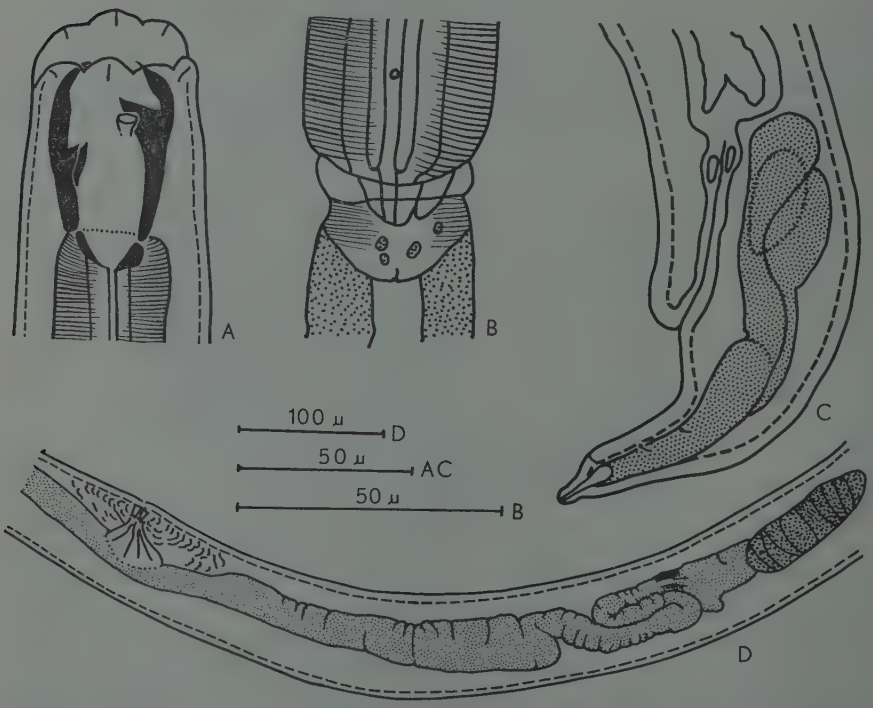

Fig. 5. *Cobbonchus chauliodus.* A. lateral view of head; B. oesophago-intestinal junction; C. female tail; D. female reproductive system.

Remarks. The name alludes to the large tooth (χαυλιοδούς = having prominent teeth).

Cobbonchus pounamua n. sp.
Figs. 6 A-I

Holotype ♀ (Awatuna) L 2.87 mm; a 44; b 3.68; c 34.4 V [27] 68.9 [15]
Allotype ♂ (Awatuna) L 2.87 mm; a 47; b 3.83; c 34.4 T 34.6

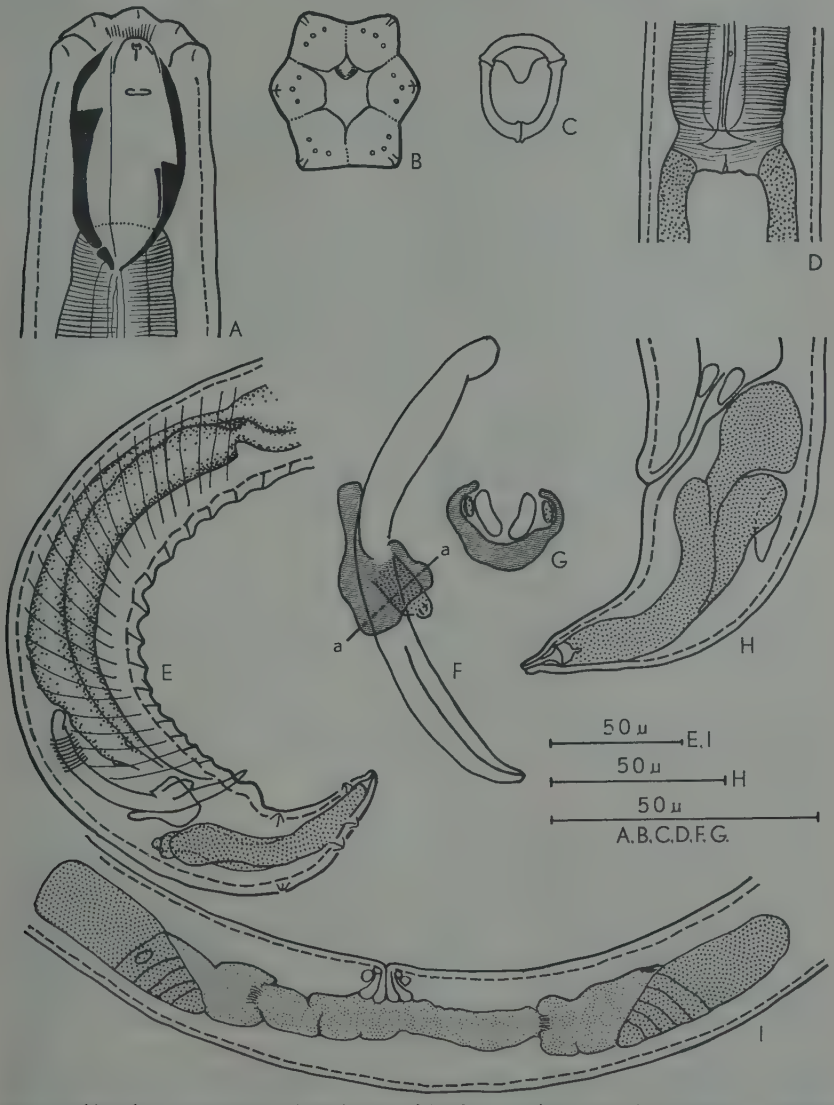

Fig. 6. *Cobbonchus pounamua.* A. lateral view of head; B. *en face* view of lip region; C. transverse section through stoma at level of dorsal tooth apex; D. oesophago-intestinal junction; E. male tail; F. spicule, lateral accessory piece and gubernaculum; G. optical section through a— a of fig. F; H. female tail; I. female reproductive system.

Paratypes ♂ (Awatuna) L 2.72 mm; a 53; b 3.76; c 30.7 T 33.5
♂ (Hokitika) L 2.17 mm, a 46; b 3.46; c 26.8 T 37.8
♂ (Fairdown) L 2.68 mm; a 46; b 4.0; c 27.9 T 32.2
♀♀ (Reefton) n = 2; L 1.26-1.30 mm; a 38-39; b 2.89-2.90; c 26.1-27.3 V [7] 61.3-63.0 [9]

Cuticle 1.5 μ thick with faint, obsolescent annulations sometimes visible under oil immersion. Subcuticle 1.5 μ thick. Lip region not offset; lateral lips more prominent than others. Amphid apertures slit-like, wide, situated a little in front of dorsal tooth apex. In *en face* view lip region hexagonal, elongated dorso-ventrally. Papillae in two rings, inner ring with eighteen papillae, three on each lip and outer ring of ten. Amphid apertures appear below outer lateral papillae as crescentic slits. At level of dorsal tooth apex the stoma is oval in cross section, with slight thickenings of the abutting edges of its skeletal elements. In lateral view stoma oval, twice as long as broad, in-arched anteriorly and pointed at base, dorsal wall thicker than ventral wall. Dorsal tooth medium sized, extending across less than a third of stoma width, apex at 31-33 per cent of stoma length, anterior edge almost straight and horizontal, posterior edge slightly concave. Subventral tooth apices at 50-55 per cent of stoma length, teeth similar to, but smaller than dorsal tooth. Posterior fifth of stoma embedded in anterior end of oesophagus. Oesophagus narrowing very slightly to nerve ring at a quarter of oesophageal length, then expanding a little to occupy three quarters body width. Oesophageal gland ducts at 52, 68-71, and 95-97 per cent of oesophageal length. Oesophago-intestinal junction non-tuberculate. Intestine, with large dark granules, passes to left of anterior ovary and to right of posterior one.

Female. Vulva a transverse slit. Vagina thick and muscular, extending across one-third body-width. Uteri paired, opposed, outstretched, short. Sphincter muscle present between uteri and oviducts. Ovaries reflexed through half length of uterus and oviduct, half as wide as body. No prerectum. Rectum straight, length equal to three-quarters anal-body-width. Tail conoid, ventrally flexed, tip with peg-like process. Annules fairly easily seen on posterior half of tail. Caudal glands massive, extending anteriorly beyond anus, ampulla conspicuous, duct terminal. A single cuticular pore near ampulla on ventral side.

Male. Testes paired, opposed, outstretched, sperm numerous. Vas deferens equal in length to testes. Opposite the second supplement vas deferens enlarges to become the ejaculatory duct, and prerectum is marked off from intestine. Spicules paired, free, slender, arcuate, and bluntly rounded at tips. Lateral accessory pieces present. Gubernaculum similar to some *Iotonchus* species; consisting of a solid dorsal part lying mostly behind the spicules from which arise a pair of lateral processes which extend forward on either side of the spicules and lateral accessory pieces and then turn inwards towards each other in front of the spicules. (Figs. 6 F & G). Supplements papilliform 16-18, largest near middle of series, decreasing in size towards either end. Tail short, conoid, ventrally arcuate, posterior

anal lip prominent. Caudal glands large, ampulla well developed, duct terminal. Cuticular pores prominent; open on tips of papilliform structures like those described in males of *Mylonchulus brachyuris* (Bütschli) by GOODEY (1942). The position of the papillae varies from specimen to specimen but is generally as in fig. 6 E.

Specific differentiation. Bears a general resemblance to *C. palustris* but differs from it in head shape, shape of stoma base, number of cephalic papillae and the orientation of the rectum.

Type slides. All type slides in nematode collection, Entomology Division, D.S.I.R., Nelson, N.Z.

Type locality. Awatuna, Westland, N.Z.M.S.1. S50/51 625672, *Ranunculus* sp. *Hydrocotyle* sp., *Juncus* sp. *Holcus lanatus* L.; soil, Mahinapua sands (wet podsolic sands).

Other localities. Reefton in sphagnum swamp; Hokitika in swampy pasture with *Juncus* L., *Drosera* L. etc.; Fairdown in swampy pasture, Westport pakihi, *Gleichenia circinata* Swartz, *Sphagnum* L., *Leptospermum scoparium* Forst. and *Drosera* L. sp.; Wharekohe *Cladium teretifolium* R. Br., *Leptospermum scoparium* Forst. and *Gleichenia circinata* Swartz. Swampy land in all cases.

Remarks. The similarity of the male tails of *C. pounamua* (the only known males in the genus) and those of *Mylonchulus brachyuris* point to a close affinity between the genera *Cobbonchus* and *Mylonchulus*. This suggested affinity is further supported by the form of the stoma with three anteriorly directed teeth set at two levels, the non-tuberculate oesophago-intestinal junction, and the similar female tails in both genera.

The specimens from Reefton are smaller than those from elsewhere and have a slightly longer oesophagus, but do not differ in other respects.

The specific name is derived from the Maori name for the region in which most specimens were collected.

I thank the Nematology Section U.S. Dept. of Agriculture, Beltsville, for permission to reproduce Cobb's drawings, and Mr. J. R. Williams for the gift of a paratype of *C. mauritianus*.

ZUSAMMENFASSUNG

Die Mononchiden (Enoplida, Nematoda) Neu-Seelands 3. Rückblick über die Gattung Cobbonchus *Andrássy* 1958 *mit Beschreibungen neuer Arten*

Die Gattung *Cobbonchus* Andrássy 1958 wurde neu definiert. Neue Beschreibungen von *C. palustris* (Cobb), *C. radiatus* (Cobb) und *C. teres* (Cobb) wurden aus COBB (1917) und einigen unveröffentlichten Abbildungen Cobbs zusammengestellt. *C. mauritianus* wird neu beschrieben. *C. chauliodus* n. sp. unterscheidet sich von anderen durch seine Grösse, den massiven Dorsalzahn und das einfache, prodelphische Ovar. *C. pounamua* n. sp. unterscheidet sich von *C. palustris* in der Kopfform, der spitzen Mundbasis und der grösseren Anzahl von Kopfpapillen.

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RECOVERY OF NEMATODES FROM INFECTED ROOTS BY MACERATION

BY

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Animal parasitologists have used enzyme digestion to separate nematodes from mammalian tissues, but so far as we know, this method has not been applied to nematodes parasitic in plants. Because various enzyme preparations and chemical methods are available to separate cells of plants, we undertook a survey of maceration procedures for the recovery of root-knot nematodes (*Meloidogyne* spp.) from root tissues. These included use of fluid from potato slices inoculated with a soft-rot bacterium, *Erwinia carotovora* (L. R. Jones) Holland, commercial enzyme preparations, HCl, periodic acid, and Jeffrey's macerating fluid.

Infected root samples were exposed to enzyme or chemical activity in Erlenmeyer flasks under constant agitation by a wrist-action mechanical shaker. The softened tissues were then beaten for about 30 minutes in a blendor with its knives replaced by rubber flails. The speed of the blendor was controlled by a variable resistance. The resulting mixture was washed with tap water through a nest of sieves. Various methods for the separation of nematodes from root fragments were explored, including decantation, flotation in sugar solutions, and differential wetting with hydrocarbons (MACFADYEN, 1955). None were completely succesful.

ENZYMES

Many pathogenic bacteria and fungi produce enzymes which attack the pectic substances that bind plant cells together (WOOD, 1959). *Erwinia carotovora,* one of the bacteria that causes soft-rot of many vegetables, has been reported to produce certain of the pectolytic enzymes that macerate plant tissues (ECHANDI, VAN GUNDY & WALKER, 1957; KRAGHT & STARR, 1953). Fluid was obtained from potato slices inoculated with *E. carotovora* maintained in culture on solid media. Half-inch slices of tubers were streaked with the organisms and kept 48 to 72 hours in moist chambers at 25°C. Rotted portions were scraped and strained through cheesecloth and centrifuged at 15,000 r.p.m. at 1° to remove bacteria and plant debris. Enough streptomycin sulfate and penicillin-G was added to the decanted fluid to give a concentration of 1000 p.p.m. Tests of activity were

made on 1 × 2-cm slices of fresh potato tuber cut 20 or 25 μ thick. These were immersed in fluid on microscope slides. The time for distintegration of the tissues gave a rough but reproducible measure of enzymatic activity. Activity of the fluid was greatest at pH 8.

The time taken to macerate various kinds of infected roots varied with the species of host plant. Samples of infected roots weighing 0.1 g were exposed at room temperatures to 20 ml of enzyme preparation at pH 8 with constant agitation. Tomato roots were reduced to fragments after 6 to 8 hours in undiluted fluid from potatoes infected with *E. carotovora,* whereas roots of *Impatiens balsamina* were fragmented in 3-4 hours. However, roots of cotton and soybean macerated more slowly than tomato.

Commercially available enzymes proved as effective as fluid from rotted potatoes. These preparations, which are derived from mold cultures, are known to contain a mixture of enxymes. Two kinds of pectinase and one cellulase [1]) macerated tomato roots, but another cellulase [2]) was ineffective. No gain in rate of maceration of tomato roots was noted when equal parts of pectinase and cellulase were combined. The most convenient product was a liquid concentrate [3]). We found it effective at 1/2 to 1/4 dilution with distilled water, adjusted to pH 4. This distintegrated tomato roots in 4 to 6 hours with constant agitation.

After exposure to the enzymes from rotted potatoes or to commercial preparations roots displayed characteristic changes. The outer tissues became translucent but the xylem vessels remained unchanged. This effect was more pronounced in fluid from rotted potatoes than in commercial enzyme preparations. The opaque white adult female nematodes became visible through the cortex and stele parenchyma (Fig. 1). At a later stage of disintegration, intact individual cells and sheets of connected cells floated in the fluid along with free nematodes and fragments of xylem vessels. Xylem elements did not disintegrate even after exposure to macerating enzymes for as long as 24 hours (Fig. 2). Intact nematodes were released from roots during maceration. They appeared in good condition, and a large percentage displayed motion of the body, of the stylet, or of the median bulb. Nematodes in all stages of development, including free eggs, were recovered from the fluid after thorough maceration of roots in the shaker. Further agitation in the modified blendor released more intact and living nematodes from softened galls. Eggs obtained in this way hatched into larvae that were infective to tomato plants. Fluid from rotted potatoes caused blackening of the egg sac within 2 hours at 25°C. This coloration appeared to be similar to the tanning described by BIRD (1958). Commercial macerating enzymes did not cause blackening of egg sacs.

CHEMICAL AGENTS

Many chemical agents have been used to soften plant tissues, especially for the

[1]) Obtained as Pectinol 10M, Pectinol 59L, and Cellulase 35 from Rohm & Haas Co., Philadelphia. Pa.

[2]) Cellulase 36, Rohm & Haas.

[3]) Pectinol 59 L.

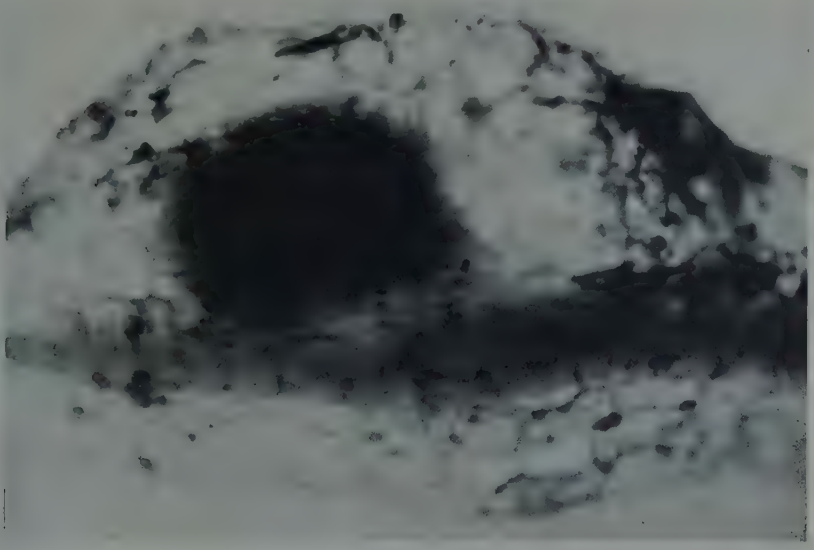

Fig. 1. Tomato root gall with adult female *Meloidogyne* showing the clearing action of commercial pectinase.

Fig. 2. Stele of squash root after maceration in commercial pectinase, showing malformations of xylem caused by *Meloidogyne*.

Fig. 3. Adult female *Meloidogyne* plus giant cells from *Impatiens balsamina* after digestion with commercial pectinase.

study of woody structures. We examined the macerating action of 1N HCl, of 0.25-2% periodic acid, and of Jeffrey's solution (10% nitric acid plus 10% chromic acid). Roots were softened by all these. Nematode cuticles were broken in HCl. Intact dead nematodes were recovered by sieving after treatment with periodic acid or Jeffrey's solution. Jeffrey's solution stained the giant cells brilliant yellow in contrast to the rest of the root.

APPLICATIONS

Solution of many problems in the biology of nematodes parasitic on plants awaits the development of suitable techniques for handling the parasites. The present investigation was designed to recover the total parasite population from a root infected with *Meloidogyne* sp. but the procedures should be useful for other purposes.

1. *Recovery of living nematodes.* Root-knot nematodes collected from infected roots digested by enzymes have remained alive in tap water for at least 96 hours. No large-scale system has yet been developed to separate the nematodes from all plant debris, but preparations clean enough for many physiological studies are easily prepared by sieving and decantation. Hand-sorting of such nematodes for biochemical work is relatively rapid. Maceration by enzymes should prove useful for physiological and biochemical studies of endoparasitic nematodes.

2. *Population census.* Both enzyme and chemical maceration were useful for sampling roots for a census of the nematode population. Blending at slow speed for 1/2 hour in the modified blendor did not destroy significant numbers of nematodes. The entire root system except xylem vessels of the larger roots was reduced to small fragments. Various size classes of nematodes were separated by sieves, which also caught small root fragments. Such mixtures may be preserved for subsequent sampling.

3. *Census of cell types.* Because large proportions of the root system are reduced to free cells, smears may be prepared for histological study. Roots macerated in commercial pectinase or in Jeffrey's solution under constant agitation yielded pieces of stele with giant cells *in situ*. After further agitation the intact giant cells were free and were recovered on the 200-mesh screen (Fig. 3). Prior fixation in formalin-propionic acid-alcohol followed by thorough washing in tap water did not alter the susceptibility of roots to maceration by enzymes or Jeffrey's solution. Thus properly fixed cells may be studied without the labor of embedding in paraffin for sectioning.

SUMMARY

A procedure for the recovery of quantities of living root-knot nematodes (*Meloidogyne* spp.) from galled roots has been developed. Infected roots are macerated by pectolytic enzymes obtained commercially or prepared from potatoes inoculated with *Erwinia carotovora*. Roots are immersed in enzymes in flasks at room temperatures with constant agitation in a wrist-action shaker. Chemical agents (periodic acid, or a mixture of chromic and nitric acids) also macerate infected roots; the nematodes are killed but their bodies remain intact. Nematodes are separated from plant debris

by washing with tap water through a nest of sieves, followed by decantation. Giant cells also may be recovered on screens. The procedure should prove useful in obtaining live endoparasitic nematodes in quantity for physiological and biochemical studies, for estimating the population in infected roots, and for the study of pathological cell types.

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OBSERVATIONS ON THE LIFE CYCLE OF *DITYLENCHUS DIPSACI* ON ONION SEEDLINGS

BY

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Ditylenchus dipsaci (Kühn, 1857) is one of the most destructive plant parasitic nematodes. Much work has been done on the host plant relationships and control of this nematode, but because of its endoparasitic habit the life cycle is difficult to follow. A review of the literature indicates that the complete life cycle of this nematode has not been described. GOODEY (1922) carried out experiments in which seedlings of red clover, about 10 days old and showing the first, true leaf, were inoculated with a single egg containing a well developed larva. Inoculation was by means of a fine glass pipette. Later examination and dissection of the seedlings revealed that sexually mature nematodes were present in from 24 to 30 days.

The life cycle of *D. dipsaci* in onion seedlings was studied by the use of inoculation techniques which made it possible to follow the daily development of the nematode. Details of the anatomy of young female and male larvae of known age also were studied.

MATERIALS AND METHODS

The inoculum used consisted of fourth-stage larvae obtained from dry garlic *(Allium sativum* L.) that had been harvested a year earlier. In all experiments sterilized pots and vials were used. Egg laying and longevity were tested in constant temperature tanks and studies of the life cycle were made in an incubator at 15° C.

Young onion *(Allium cepa* L.) seedlings approximately 15 days old with two leaves were used in all inoculations. Prior to inoculation the young seedlings grown in sterilized soil in pots were washed free of soil in tap water. They were then placed under a stereoscopic microscope and a nylon needle was introduced into the seedling parallel to its axis 2 cm above the beginning of the roots. The needle was inserted towards the root system and, after it was removed, a small amount of water was placed at the point of penetration. The needle was then reinserted at the same point and pushed back and forth several times to open the plant tissue and facilitate entrance of the eelworms. A small droplet of water was then placed on the hole and, depending upon the purpose of the experiment, various stages and num-

bers of nematodes were transferred to the droplet. After the nematodes had entered the plant tissue the needle hole was sealed with a small amount of clay. The seedlings were then planted in moist sterilized soil in glass vials 16 cm high and 4 cm in diameter, so that the point of inoculation was below the level of the soil. If the inoculation was made above the soil level, the hole was sealed with clay and the seedling kept in a moist chamber for about two hours to facilitate entrance of the nematodes and to avoid drying out of the inoculation site.

Determination of the beginning of egg deposition

Fourth stage larvae (males and females in the process of moulting) were picked from a mass population and inoculated into a seedling. After 24 hours the seedling was dissected in water in a watch glass under the microscope. By this time young mature males and females were obtained. One young female and two young males were used to inoculate seedlings which were then placed in an incubator at 15° C. At intervals of 24 hours some of the seedlings were dissected, the males and female were removed and the plant tissue stained with acid fuchsin-lactophenol and examined for the presence of eggs.

Determination of the time required for egg hatching

D. dipsaci females with eggs in the uterus were obtained by dissecting onion seedlings grown in pots containing soil infested with the nematodes. Ten females were inoculated into each of a series of seedlings. It had been observed that such females could deposit an egg within 30 minutes if kept in water. Therefore the females were inoculated into the seedling before they had opportunity to deposit eggs. Each day, beginning 96 hours after inoculation, one seedling was dissected and stained to determine the time required from egg deposition to first hatching of eggs.

By continuing the hatching experiment it was also possible to determine the time interval between hatching and the beginning of the first moult of the larvae. For this purpose one seedling was dissected daily for 10 days following inoculation with gravid young females.

RESULTS

The life cycle

Young *D. dipsaci* adult females usually begin egg deposition four days after the final moult. The time interval ranged between 3 and 7 days. Immediately after deposition eggs are deep grayish in color (Fig. 1, A) as compared to older eggs which are lighter in color. About 4 to 6 hours after an egg is deposited the first signs of cleavage become apparent. The nucleus divides and this is followed by transverse cytoplasmic division resulting in the formation of two cells. One of the cells is larger than the other. The larger cell divides and the outer cell undergoes two further divisions, one transverse and one longitudinal, to give rise to

four cells (Fig. 1, B). At this stage one end of the egg contains a cluster of four cells which later give rise to the anterior end of the larva.

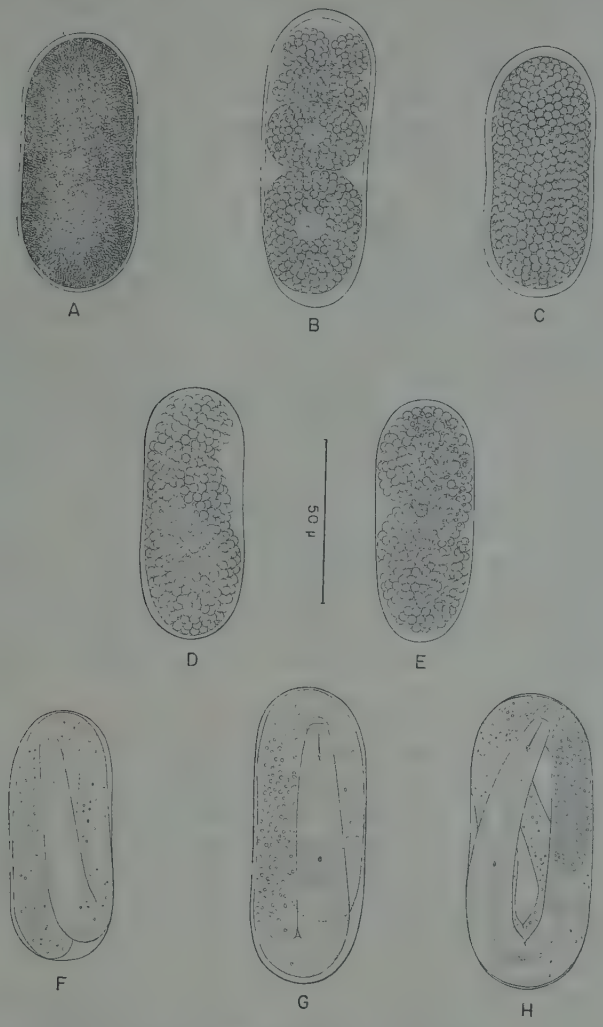

Fig. 1. *Ditylenchus dipsaci,* embryonic development. A, newly deposited egg. B, an early stage in cell division showing six cells. C, Blastula stage. D, beginning of the formation of the first-stage larva. E, very young, first-stage larva. F, full-grown, first-stage larva. G, first-stage larva undergoing moult. H, early, second-stage larva prior to hatching.

The single cell at the posterior end, formed as the result of the first cleavage, now divides. The resulting two cells together with the middle cell continue to divide. It is difficult to follow subsequent individual cell division, which proceeds

rapidly and repeatedly resulting in the formation of a large number of small rounded cells (Fig. 1, C). This may be considered to be the blastula stage. It was not possible to observe the process of gastrulation or to designate a particular stage as the gastrula.

The next observed step in the development of the larva was a sinking or withdrawing of a group of cells near one end of the egg (Fig. 1, D). This end of the egg forms the curved head region. At the posterior end of the egg there is similar development into a curved and narrower tail region (Fig. 1, E). This is the early first-stage larva and it does not exhibit any movement inside the egg. Further growth results in elongation of the body which becomes much more slender with the pointed tail region lying almost parallel with and close to the cephalic region. The cuticle has been formed by this time and there is faint evidence of the esophageal lumen a short distance behind the lip region. This is considered to be the full-grown first-stage larva (Fig. 1, F) which is reached in approximately 5 to 5-½ days from the time of egg deposition. This larva is able to move slightly inside the egg. Previously no movement was observed within the egg.

The full-grown first-stage larva moults almost immediately. The moulting process is preceded by a thickening of the anterior end of the lumen of the esophagus. This is evidently the first sign of the appearance of the stylet. Eggs containing first-stage moulting larva can be recognized under a stereoscopic microscope by the following characteristics: (a) the accumulation of dark globules inside the intestine of the larva; (b) one side of the egg containing such a larva appears to be grayish black and granulated while the other side remains whitish grey and non-granulated; (c) movements of this larval stage are not visible while movements of the second stage larva are easily seen.

Water mounts of eggs containing larvae were found to be superior to formalin or lactophenol mounts for examination under an oil immersion objective. When moulting, first-stage larvae inside eggs were examined in water mounts, the separation of the cuticle at the anterior end was easily seen (Fig. 1, G). Also, the rather slow, lethargic movements of the larvae were easily visible. It was not determined how the cuticle of the first stage was cast off. However, when moulting was complete the double cuticle at the anterior end was not present and the exuviae could be found in the fluid of the egg between the body folds of the second-stage larva (Fig. 1, H).

The minimum hatching time was 7 days at 15° C. The time from the hatching of the second-stage larva to the beginning of the second moult was 2 to 2 ½ days. Third-stage larvae began the third moult in 3 to 3½ days. Fourth-stage larvae began the fourth moult in 4 to 5 days. Adult females and males were present 9 to 11 days from the time of egg hatching. One complete life cycle took place in 19 to 23 days in onion seedlings held at a temperature of 15° C.

The sex of second-stage larvae (Fig. 2, A) could not be determined in these experiments. In third- and fourth-stage larvae sex could be ascertained by examination of the genital primordium under a stereoscopic microscope. The genital

primordium was also a useful characteristic for differentiating the various larval stages of the nematode. In the second-stage larvae the genital primordium remained unchanged without further cell division. Soon after moulting to the third stage, the anterior cells of the genital primordium were the first to begin division

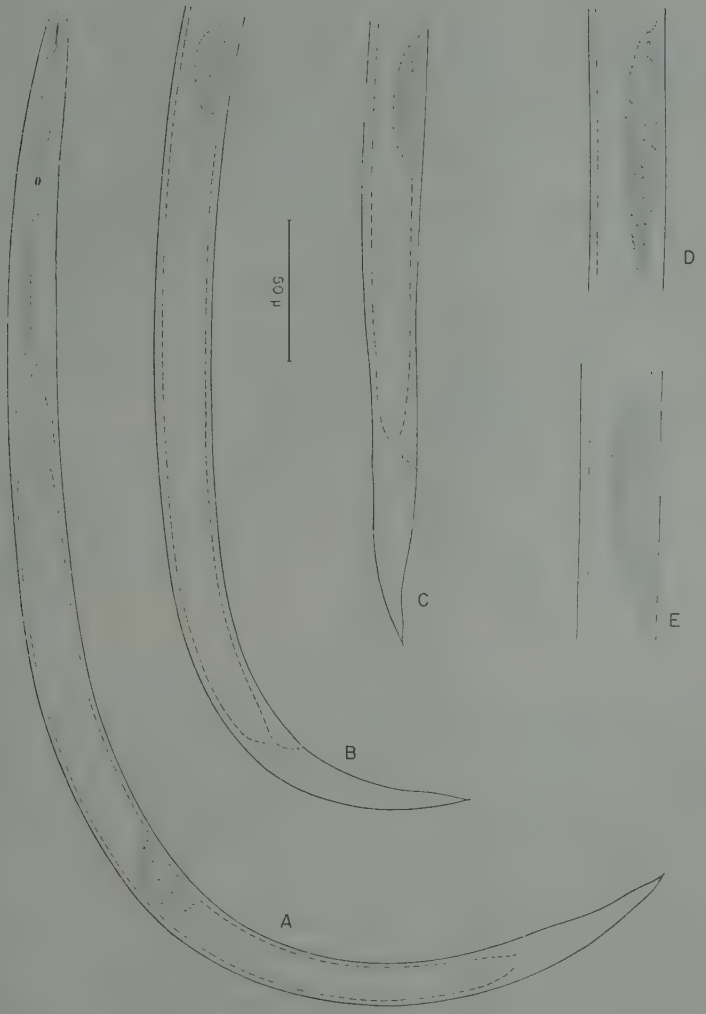

Fig. 2. *Ditylenchus dipsaci*, showing development of the genital primordium in second- and third-stage larvae. A, second-stage larva showing the position of the genital primordium. B, posterior half of young third-stage, male larva. C, posterior half of young, third-stage female larva. D, genital primordium of the full-grown, third-stage female larva. E, genital primordium of the full-grown, third-stage male larva.

if the larva was a male (Fig. 2, B). If a female the posterior cells began division first (Fig. 2, C). Cell division in the genital primordium of older female (Fig. 2,

Fig. 3. *Ditylenchus dipsaci*, showing development of gonads in the fourth-stage larvae. A, posterior half of the full-grown, fourth-stage female larva. B, posterior half of the full-grown, fourth-stage male larva. C, gonad of the young, fourth-stage female larva. D, gonad of the young fourth-stage male larva.

D) and male (Fig. 2, E) third-stage larvae continued and the anterior divisions of the males and the posterior divisions in the females could be distinguished. The genital primordium in third-stage male larvae was located midway between the posterior bulb of the esophagus and the tip of the tail. In third-stage female larvae the genital primordium was located at about two-thirds of the distance from the posterior bulb to the terminus of the tail.

The post-uterine sac began to form with young, fourth-stage, female larvae and rudiments of the vulval slit were visible (Fig. 3, C). In the early, fourth-stage male the developing gonad is composed of many cells growing both anteriorly and posteriorly (Fig. 3, D). In older, fourth-stage larvae the females (Fig. 3, A) show a developing uterus and vulval slit and, in the males, (Fig. 3, B) the spicular pouch is formed.

The body lengths of the larval stages of females do not overlap. This is also true of the male stages.

TABLE I

Measurements of adult and larval stages of D. dipsaci.
Each figure is the mean from ten individuals.

	Length in μ		
	Minimum	Maximum	Average
Second-stage larvae	327	554	412
Third-stage female larvae	597	797	724
Third-stage male larvae	595	815	700
Fourth-stage female larvae	871	1276	1079
Fourth-stage male larvae	888	1130	1013
Females	1396	1639	1503
Males	1381	1609	1464

Fecundity and longevity.

D. dipsaci females begin to deposit eggs 3 to 7 days after the final moult. Females do not deposit eggs without mating, not even sterile eggs. Thus males are necessary for reproduction in this species. A single male successfully copulated with at least three different females in one series of experiments. Females of *D. dipsaci* do not continue to lay eggs without feeding. In water a female having an egg in its uterus will deposit that egg, but no additional eggs are deposited.

The number of eggs produced by a female was determined by inoculating each of 35 onion seedlings with a single young female and two males. Thirteen to fourteen days after inoculation each seedling was carefully pulled apart along its longitudinal axis and placed in water to obtain the female and males of the original inoculation. If these were not injured they were reinoculated into a new seedling. The eggs and larvae produced by each female were counted by staining the onion seedling tissue with acid fuchsin-lactophenol. The reinoculation procedure was carried out four to six times with each group of one female and two males. It was

found that males and females of *D. dipsaci* could live in onion seedlings for 45 to 73 days and during this period of time each female produced from 207 to 498 eggs. To make an accurate determination of the number of eggs produced, the onion seedlings used for inoculation must be young. It is easier to recover the inoculated female and males from young seedlings and it is also easier to make counts of total eggs and larvae produced during the time the nematodes are in each seedling. If older seedlings are used it is difficult to extract the males and females for reinoculation without injuring them.

Females of *D. dipsaci* in onion seedlings laid eight to ten eggs each day for 25 to 30 days. Then the average number of eggs deposited by each female decreased for about 10 days. After this egg deposition ceased for 5 to 7 days. This may be a resting period to enable the female to build up stored food materials expended during rapid and extended egg laying. Egg deposition was resumed after the testing period and continued irregularly until the death.

Females of *D. dipsaci* did not deposit eggs in the green parts of old plants. However, in young onion seedlings eggs were deposited in the green parts of young succulent leaves.

This study was completed, while the author was appointed as a research fellow in the Department of Plant Nematology, University of California, Davis, California.

I thank Dr. M. W. Allen and Mr. William Nickle of the Department of Plant Nematology for offering many helpful suggestions and for reviewing the manuscript. I also thank Dr. D. J. Raski, Chairman of the Department, for his interest in my work and for allowing me the facilities of the Department.

SUMMARY

Ditylenchus dipsaci was raised on onion seedlings and its life cycle investigated in detail. A suitable technique was evolved by which the different stages of the nematode could be inoculated inside the plant tissue to observe their development.

The total duration of the life cycle ranged from 19 to 23 days, with four moults and four larval stages. The egg stage lasted 7 days, first-stage larvae inside the egg from 5-5½ days, second-stage 2-2½ days, third-stage 3-3½ days, and fourth-stage 4 to 5 days. The first moult takes place inside the egg and the hatching larva is the second-stage larva. The various larval stages are distinguishable by their total length and by the nature and location of the genital primordium. The sexes can be differentiated in the third and fourth-stage larvae as well as adults.

Mating is necessary for reproduction and the female starts deposition 4 days after the final moult. A single male was able to fertilize more than one female. Egg-laying capacity of individual females ranged from 207 to 498 eggs. Longevity of both the males and females was observed to be 45 to 73 days.

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SUSCEPTIBILITY OF VARIETIES OF CHRYSANTHEMUM TO INFESTATION BY *APHELENCHOIDES RITZEMA-BOSI* (SCHWARTZ)

BY

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In the National Chrysanthemum Society variety trials at Bell Bar, Herts, in 1958 some varieties of chrysanthemum were destroyed by eelworm whereas others were unaffected. These differences suggested that some varieties were more susceptible to eelworm than others. VOSS (1930), GOFFART (1930) and WILSON (1943) studied this aspect of eelworm disease and listed current chrysanthemum varieties according to their susceptibility, but none of these workers used uninfested controls for comparison and they did not know the size of the initial infestation on the plants. GOODEY (1933) suggested that climatic and cultural conditions might affect the severity of attack and HODSON (1933) considered that physical factors such as the size and number of stomata, hairiness of the leaf and hardness of the plant tissues might influence susceptibility. It is also possible that a variety may be regarded as susceptible because all its plants have been propagated from a single, infested source with never any attempt at eelworm control. Experiments were therefore done to ascertain whether there were true differences in susceptibility between varieties and to try to determine the reasons for them.

MATERIALS AND METHODS

Varietal susceptibility experiment

Stools of thirteen varieties of chrysanthemum were made eelworm-free by hot water treatment (46° C for 5 min.), and subsequently twenty cuttings of each variety were taken. Fifteen cuttings per variety were infested with 110 chrysanthemum eelworms of different stages using the glass tube technique (HESLING & WALLACE, 1961), and five remained uninfested as controls. The cuttings were individually rooted and potted on under glass and planted outside in May. Observations were made on the plants until they were planted out and later when they were in bloom. The degree of infestation of a mature plant was assessed from (1) the plant height (mean height of the side shoots or breaks), (2) the height of the highest infested leaf, (3) number of dead leaves, (4) number of infested leaves. To express these results as percentages the remaining healthy leaves were counted to give the total number of leaves per plant.

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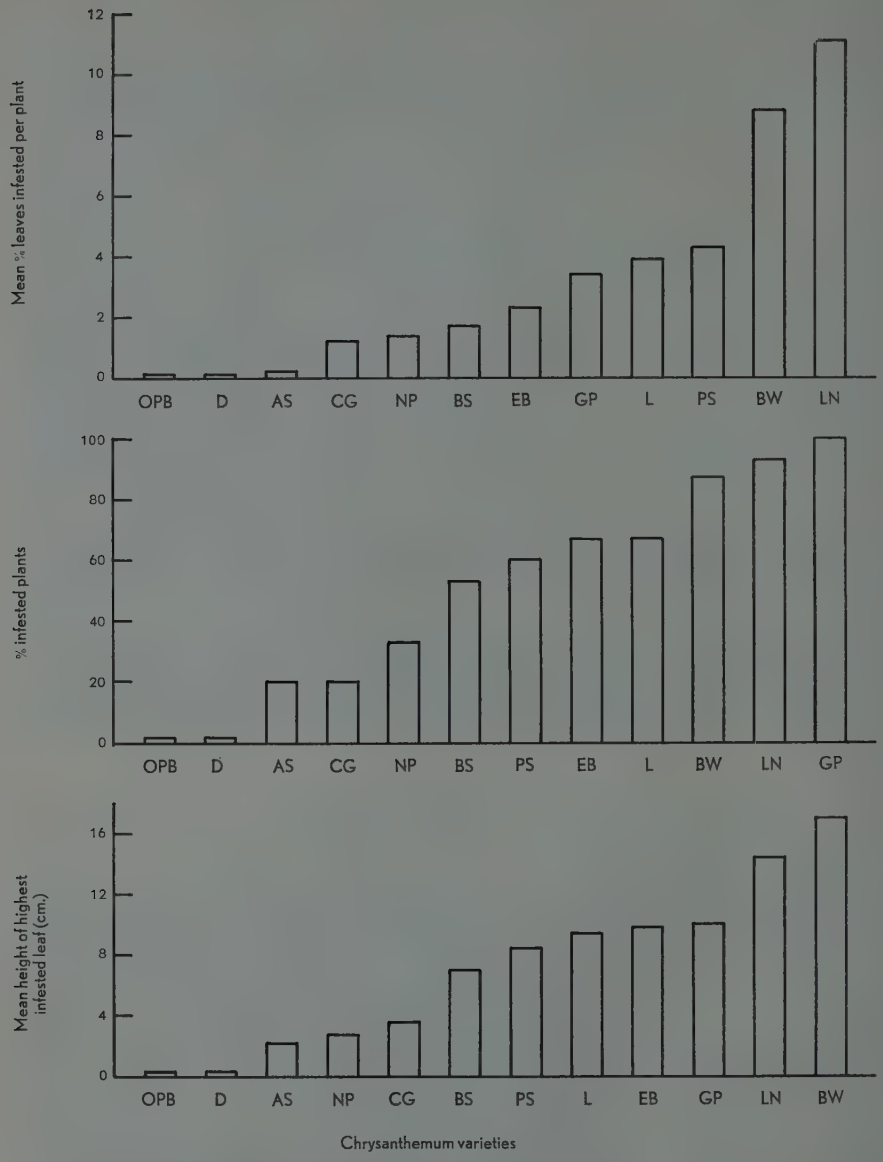

Fig. 1.. Susceptibility of different chrysanthemum varieties to infestation by *Aphelenchoides ritzemabosi*. OPB — Orange Peach Blossom, D — Delightful, AS — Amy Shoesmith, NP — New Princess, CG — Covent Garden, BS — Bill Speat, PS — Pennine Snow, L — Lucerne, EB — Evelyn Bush, GP — Greenwich Pride, LN — Lilac Nymph, BW — Bill Walters. Variety Kathleen Doward omitted because leaf discolouration could not be attributed to eelworm with certainty.

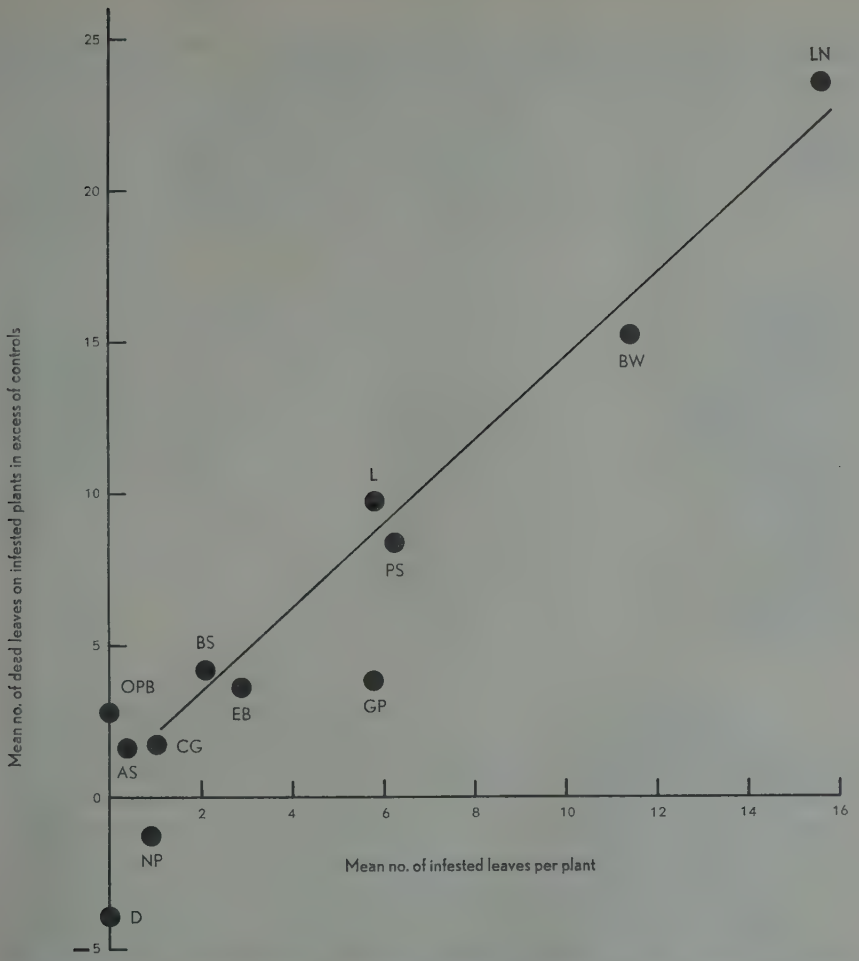

Fig. 2. Relationship between numbers of infested leaves and numbers of dead leaves on different varieties of chrysanthemums. Key to the initial letters of varieties is given in the legend to Fig. 1.

RESULTS

The rooted cuttings were measured and examined for eelworm symptoms 5 weeks after infestation. Eelworm symptoms were found in most infested plants suggesting that no variety was immune (Table I) and with one exception no symptoms were found in the controls. Some varieties showed distinct syndromes of infestation, thus variety Kathleen Doward had few discoloured leaves but much leaf distortion, whereas varieties Lilac Nymph, Delightful, Covent Garden, Evelyn Bush and Pennine Snow had many brown leaves but little distortion. About half of the infested

TABLE I

Symptoms of infestation by Aphelenchoides ritzema-bosi *in cuttings of different chrysanthemum varieties.*

Variety	Numbers out of 15 plants/variety showing: Any Symptoms	Leaf Browning	Leaf Distortion	Blindness	Ratio Mean height of controls/Mean height of infested plants
Orange Peach Blossom (12 plants)	11	10	4	5	1.9
Lilac Nymph	15	14	3	1	1.2
Lucerne	15	14	9	1	0.8
Kathleen Doward	13	3	12	6	1.7
Amy Shoesmith	14	11	7	0	1.1
New Princess	14	12	7	3	1.1
Bill Walters	15	14	9	7	1.9
Greenwich Pride	12	7	6	4	1.4
Delightful	13	12	2	0	1.1
Bill Speat	12	9	8	1	1.5
Covent Garden	13	13	2	0	1.2
Evelyn Bush	15	15	3	0	1.3
Pennine Snow	15	15	1	5	1.2

plants of Bill Walters and Kathleen Doward had a blind growing shoot but this symptom was absent in Amy Shoesmith, Delightful, Covent Garden and Evelyn Bush. Most infested plants were shorter than controls except in the variety Lucerne. No major differences in susceptibility could be found between infested cuttings of different varieties. However, there were clear differences in varietal susceptibility in mature plants. This is shown in Table II which gives the results of the examination of plants in October. Thus, Orange Peach Blossom, Delightful and Amy Shoesmith seemed resistant, whereas Bill Walters and Lilac Nymph were susceptible (Fig. 1). There were no differences in height between infested mature plants and controls, thus reduced height in infested cuttings eventually disappears. Leaf texture and susceptibility showed no correlation (Table II).

Eelworm symptoms on mature chrysanthemums usually appear first on the lowest leaves and then spread up the plant; as the disease progresses the lowest leaves die but remain on the stem. Fig. 2 shows the relationship between the number of dead leaves and the number of leaves with eelworm symptoms; it suggests that the number of dead leaves on a variety may indicate its susceptibility to eelworm and that many dead leaves on a plant may be a good indication of eelworm attack.

No correlation was found between susceptibility and stomatal size or frequency; the same result was obtained from a comparison of hair density and susceptibility. Leaf anatomy was studied in longitudinal, transverse and surface sections of leaves

TABLE II

Results of field trial to assess susceptibility of different chrysanthemum varieties to infestation by Aphelenchoides ritzema-bosi. *Variety Kathleen Doward omitted because leaf discolouration could not be attributed to eelworm with certainty.*

Variety	Mean % infested leaves/plant		% infested plants		Mean height of highest infested leaf (cm)		Mean height of plant (cm)		Leaf type
	C	I	C	I	C	I	C	I	
Orange Peach Blossom	0	0	0	0	0	0	38	30	Thin
Delightful	0	0	0	0	0	0	28	28	Intermediate
Amy Shoesmith	0	0	0	20	0	2	45	46	Intermediate
Covent Garden	0	1	20	20	1	4	31	32	Intermediate
New Princess	0	1	0	33	0	3	37	31	Thick
Bill Speat	0	3	0	53	0	7	44	41	Thick
Evelyn Bush	0	2	0	67	0	10	39	42	Thick
Greenwich Pride	0	3	0	100	0	10	27	29	Thick
Lucerne	0	4	20	67	1	9	30	33	Thin
Pennine Snow	0	4	20	60	1	8	27	24	Thin
Bill Walters	0	9	0	87	0	17	37	36	Thick
Lilac Nymph	0	11	0	93	0	15	29	28	Intermediate

C = Control. I = Infested.
15 replicates per variety.

of different size and age from the same sixteen varieties. No differences between varieties could be shown so that leaf anatomy and susceptibility could not be correlated.

DISCUSSION

The degree of eelworm infestation of chrysanthemums is probably influenced by cultural methods and by local factors such as soil type and weather; this influence may modify the susceptibility of different varieties, therefore, it seems advisable to do experiments at more than one site and in seasons with different weather. The experiments described in this paper revealed no reason for the different susceptibility of chrysanthemum varieties, but observations suggest that resistant varieties are hypersensitive to eelworm attack.

SUMMARY

Cuttings from thirteen chrysanthemum varieties were artificially infested with 110 *Aphelenchoides ritzema-bosi* of different stages. Most of the cuttings in all varieties eventually showed leaf browning or distortion or blindness. Some varieties had distinctive syndromes of infestation but there was no difference in susceptibility. Mature plants, however, showed marked differences in susceptibility to damage between different varieties. Susceptibility was not determined by stomatal size or frequency, density of epidermal hairs on leaves or stems, size of mesophyll air spaces or thickness of leaf-cell walls.

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MELOIDOGYNE KIKUYENSIS N.SP., A PARASITE OF KIKUYU GRASS (*PENNISETUM CLANDESTINUM*) IN KENYA

BY

A. DE GRISSE

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The roots of Kikuyu grass (*Pennisetum clandestinum* Höchst.) near the headquarters of the East African Agriculture and Forestry Research Organisation, Muguga, Kenya, were found to be heavily attacked by an undescribed species of *Meloidogyne* Goeldi. The galls varied in size and in the number of female root-knot nematodes, which they contained. The females were completely enclosed by the gall tissue, which resembled offset leguminous nodules to some extent.

MATERIALS AND METHODS

Infested roots of Kikuyu grass were removed from soil, washed clean and stained in cotton-blue lactophenol (FRANKLIN & GOODEY, 1949). Mature females were dissected from galled roots and their posterior cuticles cut off and mounted in pure glycerine.

Larvae and males were obtained by incubation of the infested roots at room temperature in a little water. Both were killed by heat fixed in T.A.F. (COURTNEY, POLLEY & MILLER, 1955) and stained in a weak solution of cotton blue in lactophenol. They were passed through graded concentrations of lactophenol in glycerine to pure glycerine, in which they were mounted.

Certain features of the male e.g. the lateral field were best observed when live specimens were mounted in a drop of immersion oil and examined with an oil immersion fluorite objective.

DESCRIPTION

Measurements

29 ♀♀: L = 0.72 mm (0.58-0.88); a = 1.18 (1.05-1.33); b = 0.62 mm (0.48-0.75); stylet = 15 μ (13.5-16.0).

45 ♂♂: L = 1.22 mm (0.81-1.65); a = 39.4 (28.6-44.5); b = 5.6 (3.6-7.9); c = 104.5 (70.5-149.0); stylet (27) = 18 μ (17-20); spicules (13) = 33 μ (31-35); gubernaculum (13) = 9 μ (8-11).

Holotype ♂: L = 1.65 mm; a = 41.3; b = 5.5; c = 118.0; stylet = 18.5 μ;

31 Larvae: L = 0.32 mm (0.29-0.36); a = 20.4 (17.1-23.2); b_1 = 6.7 (6.3-7.0); c = 11 (10.0-12.3); stylet = 13 μ (12-15).

65 Eggs: 0.096 mm (0.083-0.108) × 0.045 mm (0.042-0.049).

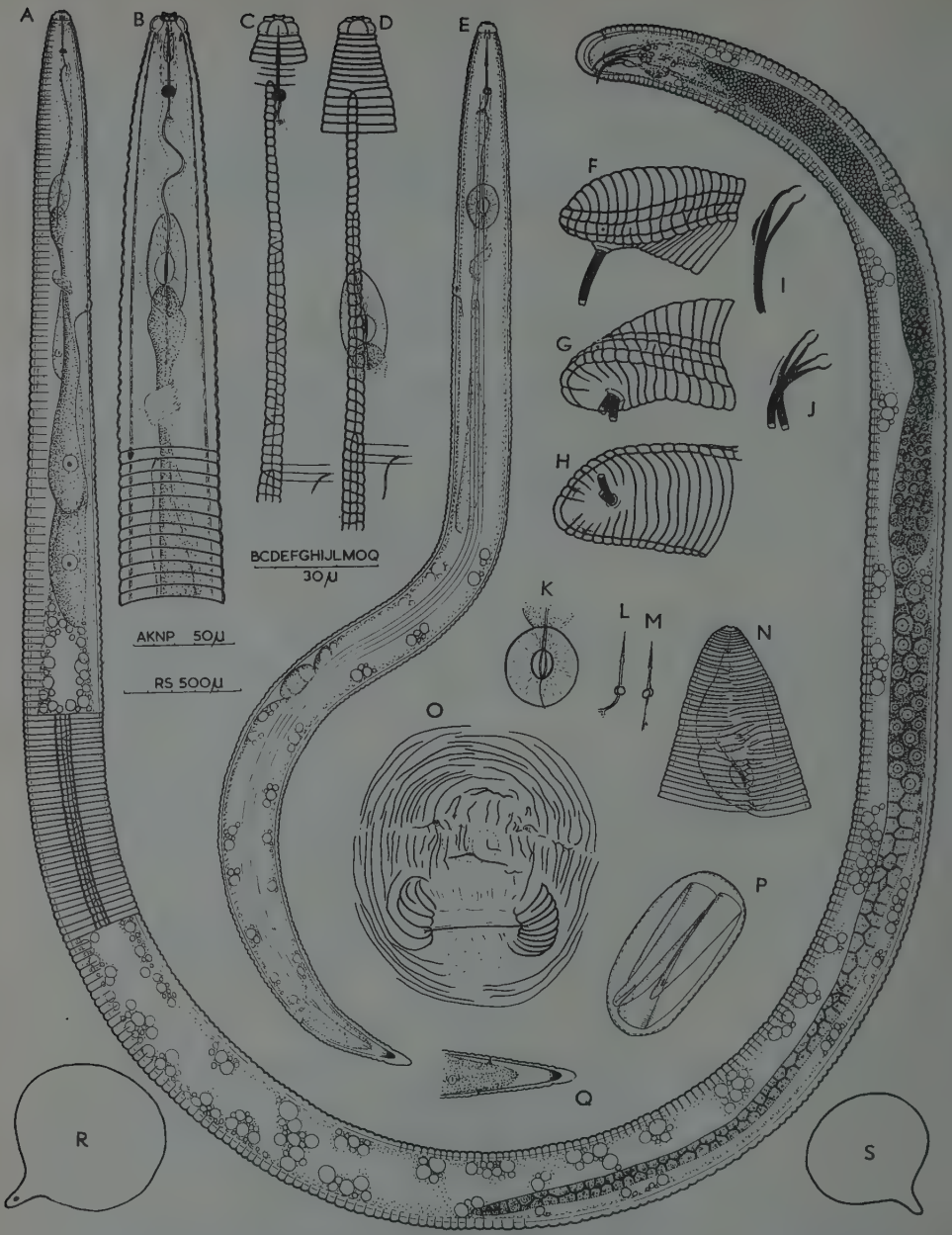

Fig. 1. *Meloidogyne kikuyensis*. A, B, C, D: Male. E: Larva. F, G, H: Tails of males. I, J: Spicules. K: Median bulb of female. L, M: Stylet of female. N: Head of female. O: Posterior cuticular pattern. P: Egg with larva. Q: Tailtip of larva. R, S: Females.

Females

Body subspherical, neck not wider than long. Body contour at posterior end smooth (Fig. 1 R-S). Head a truncated cone with two annules and a head cap. Stylet slender with rounded basal knobs (Fig. 1 L-M); anterior (prorhabdion) generally shorter than posterior section. Dorsal oesophageal gland emptying into oesophageal lumen 4 μ (3.5-5) behind stylet base. Procorpus thick, swollen, median bulb large, ovate, with crescentic valves; anterior lumen of median bulb wider than lumen behind valves (Fig. 1 K-N), wall showing two small toothlike struc-

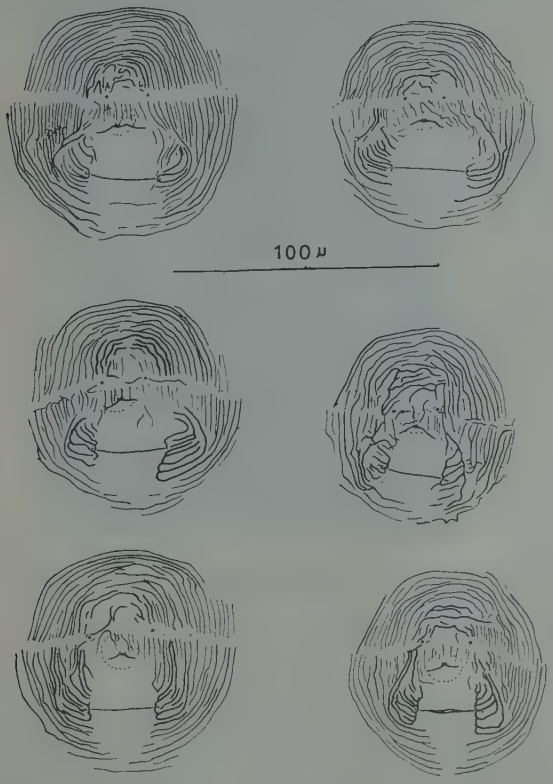

Fig. 2. *Meloidogyne kikuyensis*. Posterior cuticular patterns.

tures at different levels, halfway along the anterior lumen. Excretory pore 13 (9-19) annules behind base of head. Lateral field showing as a slight break in annules at sides of body anteriorly, and as a zig--zag line in mid-body region (Fig. 1N).

The posterior cuticular patterns had the following measurements: — Interphasmidial distance = 18 μ (14-24); Vulva width = 26 μ (21-29);

Vulva to interphasmidial line = 26 μ (17-31); Anus to interphasmidial line = 9 μ (6-11).

Posterior cuticular pattern characterised by a cheek-like structure on each side of vulva (Fig. 1,0; Fig. 2; Fig. 3) marked by prominent striae directed towards vulva. Vulva beneath general level of pattern. Immediately anterior to anus the rectum is connected to a characteristic sinus (Fig. 1,0; Fig. 2). No distinct tail whorl; tail terminus marked by irregular striae only. Anterior part of lateral fields are clear spaces unmarked by incisures, the posterior regions of lateral fields containing, in some examples, short striae; lateral fields are rarely crossed by striae. Horizontal striae are present between anus and tail-terminus.

Males

Body transversely annulated; annules 3-4 μ wide at mid-body. Lateral field commencing at about the eighth body annule (7-14), marked by two incisures (Fig. 1 C-D); at the twentieth annule (19-31) a third incisure appears in lateral field, split into two at about the 46th annule (45-49) to give four incisures

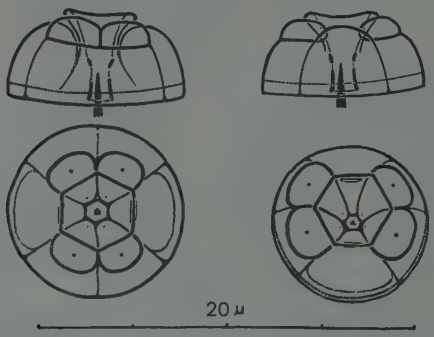

Fig. 4. *Meloidogyne kikuyensis*. Various views of male head. Top left: Dorsal. Top right: Lateral. Below: *en face*.

in lateral field for rest of its length. Lateral field then one quarter body diameter across and transversely striated over the rest of its length; the outer bands regularly, the inner band irregularly (Fig. 1A). Lateral field continues around the tail, which is always twisted (Fig. 1, F, G, H).

Head with hexagonal labial cap, partly covering large lateral and smaller subdorsal and subventral lips (Fig. 1, B, C, D, Fig. 4). Amphid apertures on lateral lips, slit-like near inner margins. Four small papillae close to mouth; subdorsal and subventral lips each with a single papilla located in middle of lip cuticle (Fig. 4). Head with two post labial annules, anterior large, posterior only about 1 μ long.

Anterior part of stylet 9 μ long (7.5-10), surrounded by tri-partite tubular

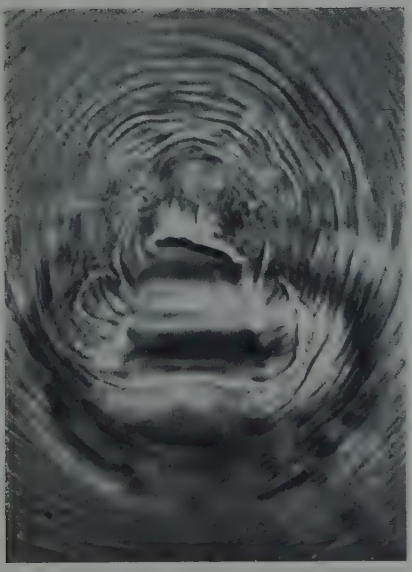

Fig. 3. *Meloidogyne kikuyensis*. Posterior cuticular pattern.

guide. Posterior part of stylet surrounded by thin muscular tube (Fig. 1, B). Dorsal oesophageal gland aperture 5 μ (4.5-6) from stylet base.

Anterior cephalids situated near the second and third body annules, posterior cephalids near eighth body annule (Fig. 1, B).

Median bulb of oesophagus elongate, twice as long as wide. Two oesophageal glands open close to the posterior end of the median bulb valves, forming a swelling which partly covers the median bulb; (Fig. 1, A, B) the dorsal oesophageal gland is small and lies on left side of the body. One bulb length behind median bulb the nerve ring encircles short isthmus.

Excretory pore 51 (45-56) annules behind the head, opposite the nerve ring. Hemizonid one or two annules or immediately anterior to excretory pore.

Phasmids situated from close behind cloaca to within about 6 μ of the tail tip; generally not opposed. Spicules (Fig. 1, I, J) equal, curved inner margins thicker than outer, spicule tips two-layered with hyaline extremities. Gubernaculum one-third spicule length with 'layering' apparent in some cases (Fig. 1 I).

Testis 51% (31-68) of body length, always single and not observed reflexed. Vas deferens full of spermatocytes and spermatozoa, ending in ejaculatory duct passing between spicules. Glandular structures found anterior to spicule heads, in some cases with ducts passing into spicules (Fig. 1A).

Larvae

Body fusiform and finely annulated. Anterior end of lateral field not observed; middle incisure appears one bulb length before median oesophageal bulb, four incisures from level of excretory pore. Lateral field 4-6 μ wide. Opposite the anus the two inner incisures are replaced by a single middle one on which, in two cases, (Fig. 1E) the phasmids appeared to lie. Phasmids not opposed, one-quarter tail length behind anus (Fig. 1Q). Tail length 2.6 anal-body-diameter. Tail tip normally smooth but sometimes annulated.

Head formed by slight constriction, with two annules and a head-cap. Stylet long and slender with rounded basal knobs. Anterior part of stylet slightly longer than posterior surrounded by a thin guiding tube. Dorsal oesophageal gland aperture 4 μ (3.5-5) behind stylet base. Rounded median oesophageal bulb 46 μ (43-49) behind headcap. Nerve ring a little more than half way between median oesophageal bulb and excretory pore. Excretory pore situated in annules 42 to 52, 66 μ (57-69) behind head. Hemizonid anterior to excretory pore.

Genital primordium situated 66%-68% of body length.

Eggs

Eggs ellipsoidal, containing larvae with three reflexures (Fig. 1P).

Type specimens

Holotype ♂ Slide No. 8 (1960-10) with measurements above; Paratype ♂♂ Slides No. 1-G (1960-10).

Type host: Kikuyu grass (*Pennisetum clandestinum* Höchst.) on roots.

Type locality: Headquarters of East African Agriculture and Forestry Research Organisation, Muguga, Kikuyu, Kenya.

Diagnosis

Meloidogyne with above characters and measurements. Distinguished from other species by the characteristic posterior cuticular pattern with low arch, lateral field without incisures, phasmids fairly close to tail end. Characteristic striated cheeklike structure near vulva and the supra-rectal sinus. Larvae differing from all species except *M. africana* Whitehead (1959), by the shorter tail. *M. kikuyensis* differs from its nearest relative *M. africana* by the complete cross annulation of the lateral field of the male and by the possession of not more than four incisures. The larvae of *M. africana* are longer and have the phasmid close to the tail terminus.

The author is grateful to Mr. A. G. Whitehead, Nematologist, E.A.A.F.R.O., for originally suggesting this study and for his advice at all stages of the work.

RÉSUMÉ

Meloidogyne kikuyensis n.sp. parasite de l'herbe *Pennisetum clandestinum* au Kénia.

Le *Meloidogyne kikuyensis* n. sp. diffère des autres espèces du genre *Meloidogyne* par la forme caractéristique de la partie anale chez les femelles. Le patron cuticulaire postérieur montre un arc dorsal bas, plus ou moins carré, des joues typiquement striées de chaque côté du vulve et une figure sinussoidale entourant l'anus en dessous du cuticle. Les larves diffèrent de toutes les autres espèces excepté de *M. africana* et *M. inornata*. Le mâle de *M. kikuyensis* diffère de *M. africana* et *M. inornata* (Lordello, 1956) par le quadrillage sur toute la longeur des côtés latéraux et par le nombre constant des raies qui est toujours de quatre.

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STUDIES ON THE HOST-PARASITE RELATIONS OF *PRATYLENCHUS PENETRANS* (COBB) TO APPLE SEEDLINGS

I. PATHOGENICITY UNDER STERILE CONDITIONS [1])

BY

R. S. PITCHER [2]), Z. A. PATRICK [3]), W. B. MOUNTAIN [3])

In the past many authors have associated *Pratylenchus penetrans* (Cobb) with poor root growth, decline in vigour and replanting problems of apple. Much of the evidence has been circumstantial and dependent upon correlations between nematode numbers and the severity of symptoms or on improvements brought about by soil fumigants (BOSHER & NEWTON, 1957; MAI, DOLLIVER, KIRKPATRICK & PARKER, 1957; OOSTENBRINK, 1955 and 1956). The complexity of field conditions make controlled inoculation the only satisfactory method of measuring the effect of *P. penetrans* on apple growth but, as yet, very few experimental inoculations appear to have been carried out. Those of MAI (1960) and OOSTENBRINK (1956), who employed not more than four replications of one high dosage of unsterile nematodes, showed marked growth depression. DECKER (1960) also showed growth depression whenever his inoculum became established. However, in pot trials carried out by the present authors (unpublished) the inherent variability of plant and nematode material has made it difficult to show any significant growth depression, even with a range of dosage of surface-sterilised nematodes and ten- to twelve-fold replication. Without strict control of experimental conditions it is also impossible to determine whether the nematode acts as a primary parasite or as an incitant which produces infection courts for other organisms. The object of the following study was to investigate the reactions of apple to *P. penetrans* under sterile conditions and to compare them with those reported by MOUNTAIN & PATRICK (1959) for peach. Such a study was considered an essential first step in resolving the role of the nematode in the field disease complex.

METHODS AND MATERIALS

Experimental material was prepared as follows:

Apple seedlings — seeds were taken from fresh, ripe fruits of the varieties McIntosh or Worcester Pearmain and air-dried at room temperature. As required,

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[1]) Joint contribution from the East Malling Research Station, England, and contribution No. 22. from the Research Station, Research Branch, Canada Department of Agriculture, Harrow, Ont.

[2]) East Malling Research Station.

[3]) Research Station, Harrow.

batches of seed were moistened with 50% alcohol for 3 minutes, then immersed in 0.1% mercuric chloride solution under reduced pressure for 5 minutes, and finally transferred to a mixture of two fungicides containing 75 ppm. ethoxyethyl mercury chloride and 0.19% thiram. The seeds were then transferred singly to 15 × 150 mm tubes filled with moist washed sand to a depth of about 40 mm, such tubes having been filled, plugged and autoclaved for 40 minutes at 15 lbs. pressure before use. The seeds were then matured by storing the tubes in the dark at 3° C for about 70 days. As required, seeds were removed from the tubes and resterilised by immersion in a solution of 2% chloramine T and 0.01% sodium dioctylsulphosuccinate for 20 minutes at reduced pressure. After three washes in sterile distilled water, they were replanted singly in 25 × 250 mm tubes containing about 80 mm depth of sand moistened with an inorganic nutrient solution, essentially that of HOAGLAND & ARNON (1950), iron citrate or chelated iron being added at treble the recommended rate. All tubes were filled, plugged and autoclaved for 40 minutes at 15 lbs. pressure before use. After sowing they were kept in a dark room at 13° C until germination began; they were then transferred to a growth room with an air temperature of 22° C during illumination and 13° C during darkness. The tops of the plants received approximately 1200 foot-candles of artificial illumination for 16 hours daily and the roots were kept dark by plunging the base of the tubes in trays of sand placed in a soil temperature tank at 13° C. After 3 to 4 weeks, when the plants were about 80 mm high, those to be inoculated were selected at random, the remainder serving as uninoculated controls.

Peach seedlings — these were prepared as described by MOUNTAIN & PATRICK (1959).

Nematodes — inoculum free from culturable fungi and bacteria was prepared and an aliquot containing approximately 1000 *P. penetrans* pipetted under sterile conditions into each tube containing an apple seedling. After a requisite period in the growth room, tubes were broken open under sterile conditions and small pieces of root checked for microbiological sterility by plating on a variety of media. (See MOUNTAIN & PATRICK (1959) for details of the above techniques). The remainder of the root system was washed, examined under a stereoscopic microscope, stained in boiling cotton blue-lactophenol and cleared in phenol (FRANKLIN, 1949). The stained root sections were examined microscopically for the number of nematodes present, their location and the reaction of the root to their presence. Sections of root were also examined and in most cases the sand was screened and the nematodes counted.

RESULTS

Only roots shown to be free from culturable bacteria or fungi were used for the following observations. With the help of a stereoscopic microscope the apple roots were seen to react in two ways to invasion by nematodes. There was firstly a yellowing of groups of up to ten cells in the epidermal region. Through the

glass wall of the tube individual nematodes could often be seen either lying on or embedded amongst the discoloured cells. This reaction could be detected within 3 hours of inoculation, but after several days the discolouration tended to fade. By this time the nematodes had either moved away from the discoloured areas or had entered the cortical cells. Little more could be seen through the wall of the tube until 3 or 4 weeks had elapsed. Then, here and there, dark streaks could be detected in the region of the stele, but the overlying cortical cells appeared not to be affected. This reaction differs sharply from that of some other hosts e.g. peach (MOUNTAIN & PATRICK, 1959) or *Delphinium* (STONE, 1953) in both of which cortical lesions occur rapidly.

Before giving a fuller account of these reactions a brief description of the anatomy of the young apple roots under consideration may be of advantage. The

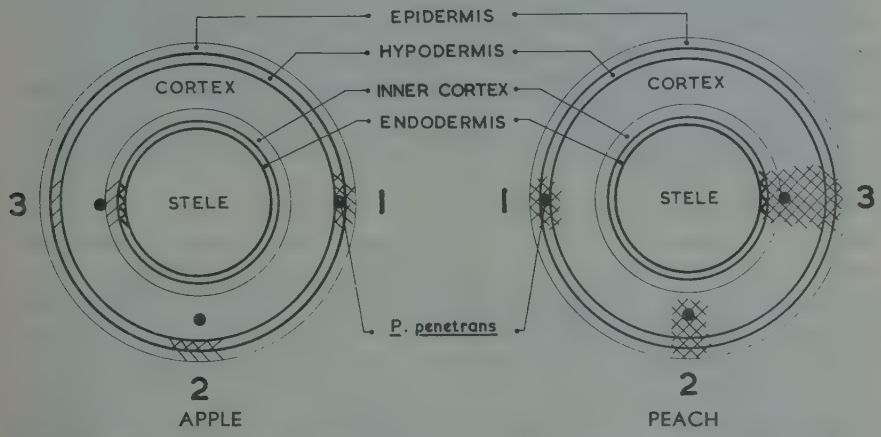

Fig. 1. Diagrammatic transverse sections of young roots of apple and peach, showing normal structure and successive stages (1-3) of injury caused by *Pratylenchus penetrans*, under microbiologically sterile conditions.

roots examined were 1 to 2 months old and virtually devoid of secondary thickening, the diagrammatic transverse section in Fig. 1 shows the main features. The outermost layer, the epidermis, consists of small cells, most of which bear a root hair. Beneath this is the hypodermis, a single layer of larger, thicker-walled cells, fairly long in proportion to their cross-section and closely-packed, without intercellular spaces. The epidermis and the hypodermis will be referred to collectively as the dermal layers. Then follows a ring of cortical parenchyma five to ten cells deep, loosely-packed, thin-walled and variable in size and shape. Immediately outside the endodermis (a single layer of regular, thick-walled cells) is a layer of specialized inner cortical cells, also thin-walled, but bearing conspicuous strips of thickening on their anticlinal walls. Such a cell layer is commonly present in woody Rosaceous plants (ESAU, 1943; RIEDHART & GUARD, 1957) and has often

been confused with the endodermis because its wall thickenings are mistaken for Casparian strips. True Casparian strips are present in the endodermis of apple but are obscured at an early stage by suberisation of the cell walls. The endodermis and the inner cortical layer will be referred to collectively as the endodermal layers. Between the endodermis and the vascular cylinder lie two to four layers of parenchymatous pericycle cells. The anatomy of young peach roots is essentially the same as that of apple.

Regardless of the number of nematodes involved, the pattern of plant reaction was consistent; nematodes penetrated the epidermis in a radial direction, probably intercellularly, but on reaching the hypodermis they usually aligned themselves with the longitudinal axis of the root and moved along by penetrating the transverse walls of successive cells (Fig. 2). The contents of invaded cells and of some of those adjoining became disorganized and much of the cytoplasm disappeared or retreated, together with the nucleus, against the cell wall. The cell walls and/or adherent cytoplasm took on a light brown colour, and were thereby rendered visible as small yellowish spots under a low-power microscope. From the hypodermis the nematodes usually moved into the cortex (Fig. 3) although in some cases they seemed to be unable to do this and left the root by way of the epidermis. Many nematodes remained to feed and reproduce in the mid-cortical cells, which showed little visible injury or reaction for at least a month. Other nematodes, however, moved quickly to the deeper layers of the cortex and aligned themselves with the longitudinal axis of the root, just outside the endodermis. Nematodes have been seen to penetrate the inner cortical layer (Fig. 4) but not the endodermis, which seemed to be a barrier to them, as it is to fungi in some other hosts (Talboys, 1958; Voorhees, 1934). The endodermal layers responded within 24 hours to the presence of nematodes in much the same way as the dermal layers. The endodermis, however, reacted much more sharply than any of the other tissues. The brown discolouration was deeper and more permanent and, as feeding progressed, this discolouration extended to large groups of cells, often becoming visible to the naked eye (Figs. 1, 3, 4). Although here and there small batches of cortical parenchyma became lightly discoloured, the bulk of this tissue remained remarkably healthy, even when packed with adults, eggs and larvae. This suggested some fundamental biochemical difference between the cortical parenchyma on the one hand and the dermal and endodermal layers on the other. In histochemical tests in which freshly-cut transverse sections of apple roots were immersed in diazotized sulphanilic acid or diazotized *p*-nitroaniline solutions, a yellow to brown colour reaction occurred very rapidly in the cell walls and contents of the dermal and endodermal layers. The cortical parenchyma, however, reacted only feebly to either reagent. Both substances caused a faint yellowing of the contents of some cells but the cell walls responded only to *p*-nitroaniline. These reactions were interpreted to indicate the presence of relatively large concentrations of phenolic substances in the dermal and endodermal layers of the young apple roots and considerably smaller amounts in the cortical

Fig. 2. Hypodermal cells of apple root, penetrated and disorganised by *Pratylenchus penetrans.*

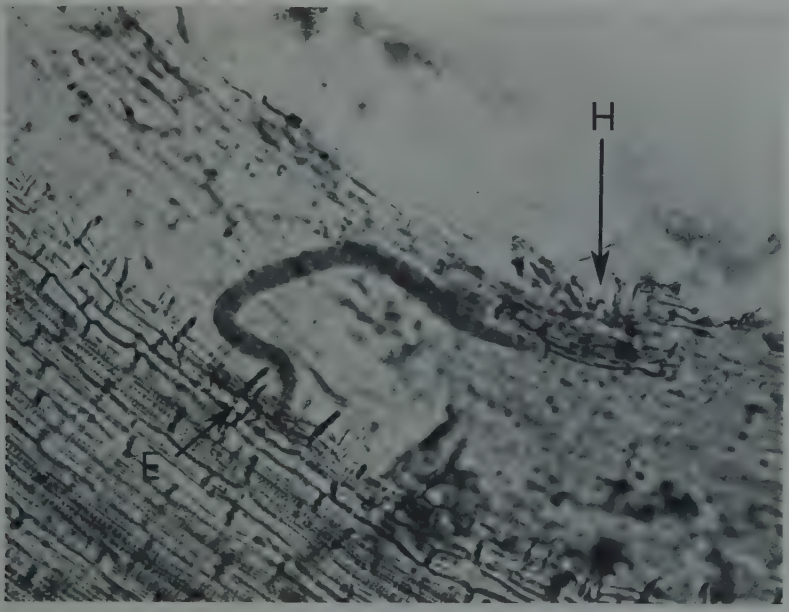

Fig. 3. Apple root invaded by a single nematode (*P. penetrans*), showing discolouration of hypodermis (H) and endodermis (E) but not of cortical parenchyma. 24 hours after inoculation.

Fig. 4. Transverse section of apple root showing discolouration of endodermis (E) in vicinity of nematodes (*P. penetrans*), one of which has penetrated an inner cortical cell. 11 days after inoculation. N — nematodes.

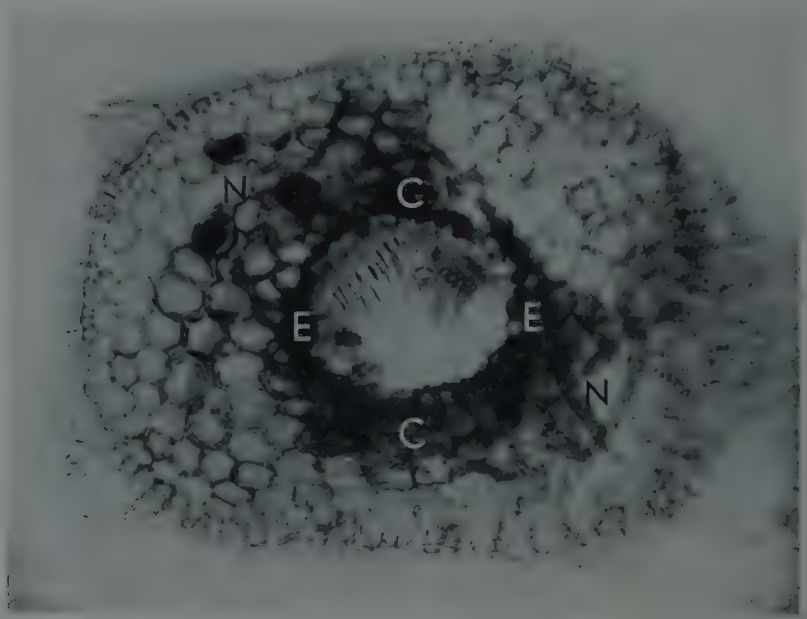

Fig. 5. Transverse section of peach root, showing discolouration of cortex (C) and endodermis (E) in vicinity of nematodes (*P. penetrans*). 6 days after inoculation. N — nematodes.

parenchyma. Comparable tests carried out on sections of peach roots showed an immediate yellow colour reaction in the cortical parenchyma, as well as in the dermal and endodermal layers. It would appear therefore that, in both apple and peach roots, the tissues that show a rapid discolouration in the presence of *P. penetrans* also contain relatively large amounts of phenolic substances.

DISCUSSION

The above observations show that *P. penetrans* is a primary parasite of apple feeder roots because it can invade, feed on and reproduce in them in the absence of all other organisms. The nematode is also pathogenic to apple for it regularly causes discolouration and necrosis of the dermal and endodermal root tissues. However, apple is a less sensitive host than peach because it can sustain *P. penetrans* in its cortex for periods of a month or more without suffering serious injury, whereas in peach the cortex shows discolouration and necrosis within a few hours of invasion (Fig. 5).

The reasons for such a marked difference in the sensitivity of the two hosts to parasitism by *P. penetrans* are no doubt complex. As there are no apparent differences in the penetration and subsequent colonization of the nematode in either host, it is quite possible that much of the difference in sensitivity is due to host-induced reactions. The observed differences in the concentration and distribution of phenolic substances in the root tissues of the two hosts may be the most significant factor involved in the mechanism of lesion formation. The histochemical tests suggest that in apple roots the cortical parenchyma, which is quite tolerant to nematode colonization, is relatively free of phenolic substances which react with the reagents used. On the other hand, the dermal and endodermal layers, which show a rapid necrotic reaction to *P. penetrans,* contain high concentrations of these substances. In contrast however, similar tests carried out on peach root sections showed that the concentration of phenolic substances is high in all tissues. A feasible explanation is provided by the findings of MOUNTAIN & PATRICK (1959) that *P. penetrans* is capable of breaking down amygdalin under sterile conditions, probably by means of a β-glucosidase enzyme, thereby releasing phytotoxic substances. Although apple roots contain no amygdalin, β-glucosidase enzymes are active against other phenolic substances, the break-down products of which could cause the discolouration and necrosis noted in the dermal and endodermal layers of apple.

Therefore, in both peach and apple the extent of root necrosis resulting from nematode attack appears to depend on the presence of specific chemical plant constituents which, when acted on by the nematode's enzymes, give rise to products that are toxic to invaded and adjacent host cells. The type and extent of the lesion produced may therefore largely depend on the type and distribution of such chemical constituents in the root.

The major part of this work was carried out in the Nematology Section of the Research Station, Canada Department of Agriculture, Harrow, Ontario. The first

author wishes to thank the Canada Department of Agriculture for generously providing a supplementary allowance, East Malling Research Station for leave of absence, the Agricultural Research Council for meeting travelling expenses and, finally, the Director, Dr. L. W. Koch and members of staff at Harrow for their hospitality, the provision of facilities, their ready cooperation and many kindnesses. He also wishes to acknowledge specialist help and advice given by several colleagues at East Malling Research Station.

SUMMARY

A technique is described for the inoculation, under aseptic conditions, of apple seedlings with the root lesion nematode *Pratylenchus penetrans* (Cobb, 1917) Filip. & Stek., 1941. Under these conditions apple feeder roots (i.e. those without secondary thickening) react to nematode invasion by a rapid discolouration of the outermost, (i.e. epidermis and hypodermis) and innermost, (i.e. inner cortex and endodermis) cortical tissues, but show little or no reaction in the intervening cortical parenchyma. This response is compared with that of peach roots, in which all the cortical tissues are readily discoloured by this nematode. Histochemical tests suggest that sensitivity to *P. penetrans* is correlated with the presence and concentration of phenolic substances in the various tissues of these two hosts.

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OBSERVATIONS ON THE BEHAVIOUR OF *APHELENCHOIDES RITZEMA-BOSI* IN CHRYSANTHEMUM LEAVES

BY

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Recent work on the epidemiology of chrysanthemum eelworm *Aphelenchoides ritzema-bosi* (Schwartz) has stressed the importance of methods of spread (HESLING & WALLACE, 1961 a), the recognition of symptoms (HESLING & WALLACE, 1961 b), varietal susceptibility (HESLING & WALLACE, 1960) and factors influencing eelworm migration on the plant (WALLACE, 1959). There is, however, little information on eelworm activities within the leaf. This information is necessary before such problems as the chemical reactions of the leaf to infestation and the nature of resistance can be studied. This paper deals with the life-cycle, distribution and movement inside the leaf and compares the activities of the early and late stages of the eelworm.

MATERIALS AND METHODS

Eelworms were extracted in a mistifier apparatus (SEINHORST, 1950) from infested leaves which had been dried slowly and stored in the laboratory. Leaves were artificially infested by placing a known number of eelworms in a drop of water on the undersurface. The water drop was covered with a piece of polythene, 2 cm by 1 cm and held in place with a paper clip which ensured that the water drop did not run off the leaf nor evaporate quickly and also spread the eelworms over a greater surface area.

The distribution and numbers of eelworms in green and slightly discoloured parts of infested leaves were obtained by staining the leaf in acid fuchsin — lactophenol (FRANKLIN, 1949). Brown parts of the infested leaf were macerated in an Atomix 100 homogeniser for 15 seconds, which broke up the leaf tissues but left the eelworms intact for counting.

RESULTS

Life-cycle

STEWART (1921) states that the length of life-cycle of *A. ritzema-bosi* is about 15 days, but the experimental conditions were not stated. To study the life cycle, forty healthy chrysanthemum leaves of variety Pennine Snow were each infested with five gravid females. The leaves were inserted by their petioles into damp

peat in seed boxes and covered with polythene at a temperature of 14°-17° C. Variety Pennine Snow browns very slowly when infested with eelworms and shows no symptoms after 16 days. Eelworms in the leaves can, therefore, be examined *in situ* by staining the leaves in acid-fuchsin — lactophenol. Every 2 days for 16 days five leaves were removed from the boxes and stained in this way and adults, larvae and eggs counted. The results (Fig. 1) indicate that there is no increase in the original number of adults until 10 to 13 days after infestation. The first eggs

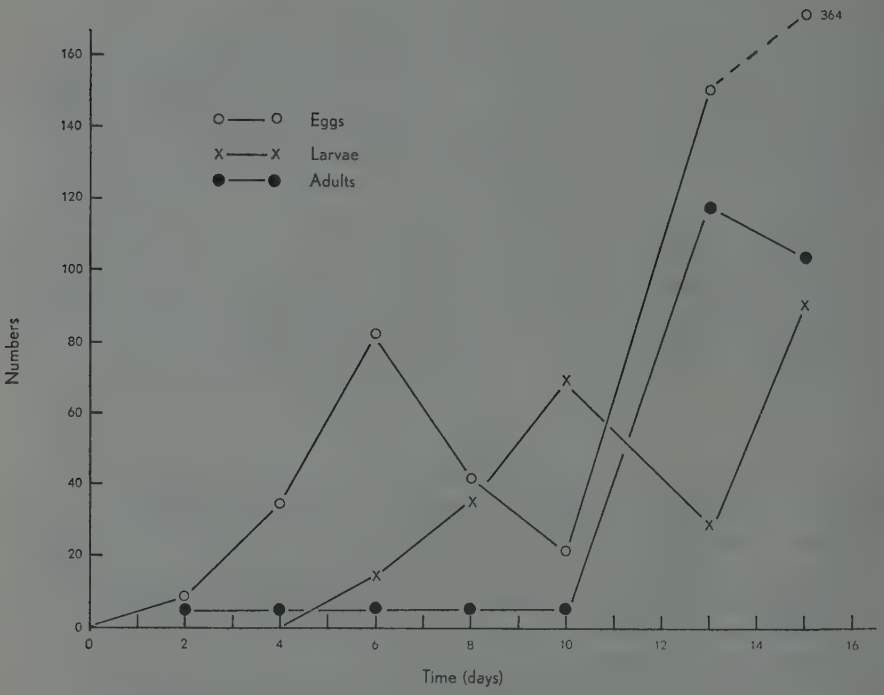

Fig. 1. Numbers of eggs, larvae and adults in chrysanthemum leaves at various times after infestation with five gravid females per leaf. Each point is the mean of five replicates.

were found 2 days after infestation and their numbers rose to a maximum after 6 days. The number of eggs then fell until 10 to 13 days after infestation, when there was a marked increase as the new adults laid eggs. The larvae showed similar fluctuations. The data show that the length of life cycle is about 10 to 13 days.

Examination of the stained infested leaves showed that each female lays about twenty-five to thirty-five eggs in a compact group. The eggs take 3 to 4 days to hatch and the larvae about 9 to 10 days to reach maturity. Adult females may lay eggs within 1 or 2 days after reaching maturity.

Distribution in the leaf

The first external sign of eelworm infestation is a small brown-green patch on the leaf. The discolouration spreads until the whole sector between the leaf veins becomes brown. Infested leaves were cut up to determine the distribution of the eelworms in relation to leaf discolouration. Several leaf sectors in a partial stage of browning were selected and cut into dark brown, brown-green and green portions, each of which was then treated and the eggs, larvae and adults counted. Ten sectors of similar size and stage of browning from leaves of variety Lilac Nymph were examined and the results are shown in Fig. 2. In the dark brown portions there were relatively few eelworms of any stage although adults predominated. In the brown-green portions there was a preponderance of adults

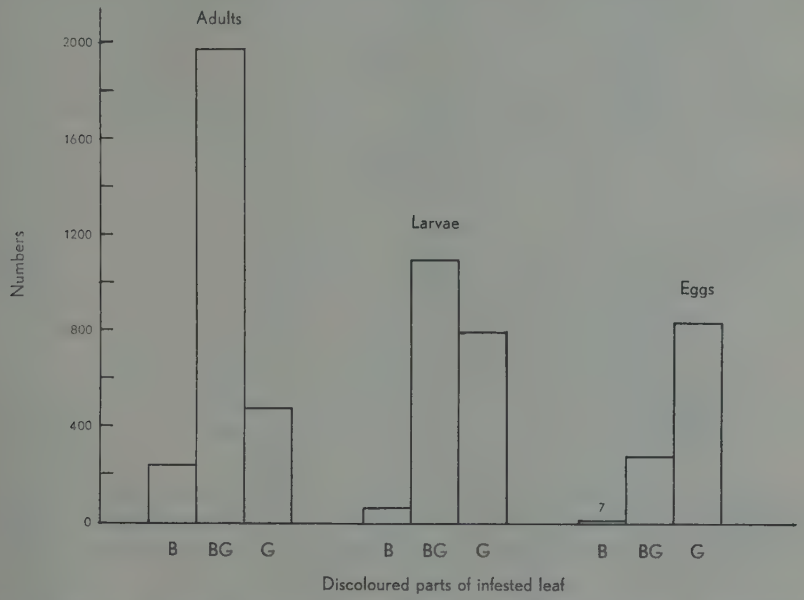

Fig. 2. Distribution of eggs, larvae and adults in partially discoloured sectors of infested chrysanthemum leaves. B — Brown; BG — Brown-Green; G — Green.

and larvae with few eggs. The green parts of the sector contained many eggs and larvae and few adults. These gradients of distribution of the three stages suggest that fertilisation and egg laying occur chiefly at the boundary between the brown-green and green parts of the infested leaf sector. Egg laying appears to be associated with regions where there is sufficient food supply.

Movement inside the leaf

The brown-green parts of infested leaves are nearly transparent, so the eelworms inside the leaf can be observed directly under the binocular microscope

with transmitted light. Examination of numerous infested leaves showed that when the leaf was dry, eelworms inside the leaf moved only slightly. On wetting, water entered the leaf and filled the mesophyll air-spaces of the discoloured areas and spread about 1 to 5 mm into the green parts. The eelworms then moved actively, many emerging on to the leaf surface through the stomata. When infested leaves were removed from plants on a dry day and dissected under water, the eelworms removed from the leaf were inactive but most began to swim after about 1 minute. Similarly, examination of leaves which had been wetted for several hours by rain showed that most of the eelworms were active when removed

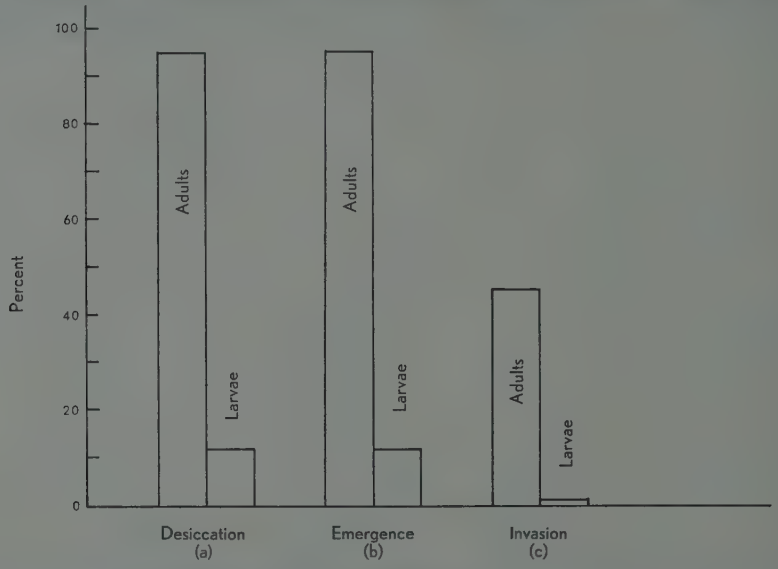

Fig. 3. (a) Per cent of adults and larvae surviving three days drying in infested leaves; (b) Per cent of adults and larvae emerging from leaves in water in two days; (c) Per cent of adults and larvae invading a leaf in two days.

from the leaf. Eelworms require a water film in which to move, thus, it seems likely that fluctuations in weather conditions influence the water content of leaves and eelworm activity. This may partly explain why the spread of eelworm infestation in leaves is most rapid during the late summer and autumn when mists and low temperatures produce high moisture in the leaves for long periods.

A COMPARISON OF THE ACTIVITIES OF ADULTS AND LARVAE

Resistance to desiccation

Infested chrysanthemum leaves which are dried and stored for one year still produce active eelworms. This ability to resist drying has been described by many authors but there is no information whether all stages have this property. The

brown-green portions from five infested leaves were each macerated and the eelworms counted and classified as either small early stage larvae, late stage larvae, or adults. Similar leaf tissues were left to dry for 3 days and then macerated and eelworm counts taken. The results (Fig. 3a) show that 95% of the late stage larvae and adults survived drying compared with 12% of the early stage larvae. Most of the larvae recovered from the dried leaves were shrunken, distorted and dark in colour.

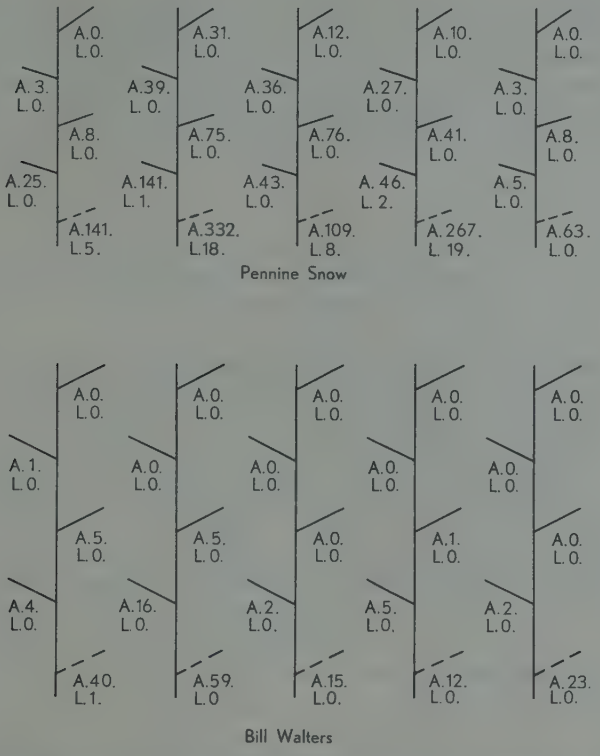

Fig. 4. Distribution of adults and larvae on the surface of leaves on two varieties of chrysanthemums. The highest leaf showing symptoms (dotted line) and the four leaves above without symptoms (continuous lines) were sampled. A = adults, L = larvae. The difference in leaf size and length of internode between the two varieties is shown diagramatically.

Emergence from leaves

Infested chrysanthemum leaves were immersed in water for 2 days and the early stage larvae and late stage larvae and adults which emerged were counted. The leaves were then macerated and similar counts were made of eelworms which had not emerged. The results (Fig. 3b) indicate that about 95% of the late stages emerged compared with 12% of the early stages.

Ten infested leaves were removed from plants after a period of heavy rain and washed to remove any eelworms on the surface. The mean number of late stages per leaf was 182 compared with 9 early stage larvae. This observation also shows that few of the early stage larvae emerge from the leaf.

Invasion of leaves

A suspension of all stages of chrysanthemum eelworm was prepared by teasing out infested leaves in water and then washing through a sieve (eighty meshes/inch) to remove plant material. Five leaves of variety Pennine Snow were each infested with about 60 late stage and 60 early stage eelworms. After 2 days the leaves were stained in acid-fuchsin—lactophenol and the adults and larvae within the leaf were counted. The results (Fig. 3c) show that of the late stages introduced on to the leaf surface 45% invaded whereas only 1% of the early stages did so.

Distribution on the plant surface

The numbers of early and late stage eelworms on five leaves on each of five plants in two varieties of chrysanthemum were obtained by leaf washing. The highest leaf showing eelworm symptoms and the next four leaves above it on each plant were examined in this way. The data (Fig. 4) show that in only two plants were early stages recovered above the highest leaf showing symptoms whereas late stages were present on all the plants. This suggests that the upward migration of early stages is slight, supporting previous findings by WALLACE (1959). Varieties Pennine Snow and Bill Walters differ in leaf texture, leaf size, number of branches and flowers and length of internode. Pennine Snow has a small thin leaf with numerous branches and flowers and short internodes. Bill Walters has opposite characteristics. The compact bushy nature of the Pennine Snow plant helps to maintain moisture on the leaves and stems longer than on Bill Walters, which may explain why there are more eelworms on the leaves and a greater upward migration on Pennine Snow.

Movement

The wave frequency, speed and body length were determined for adults, intermediate size larvae (stages three and four) and small larvae (stages one and two). Twenty individuals were observed in each category and the results are given in Table I.

TABLE I

	Mean wave frequency (waves/min)	Mean speed (mm/min)	Mean length (μ)
Adults	102	20	880
Intermediate larvae	42	5.1	550
Small larvae	16	0.5	230

The greater length and wave frequency in adults produce greater propulsive forces and consequently higher speeds. Thus, adults are capable of swimming in thick water films whereas larvae only progress when in contact with the substratum. Ability to emerge from and invade leaves and to migrate up the plant is probably determined by the propulsive force generated by the eelworm, hence the low activity of early stage larvae.

DISCUSSION

Many adults emerge from the brown sectors of infested leaves after rain and migrate up the stem to other leaves which they invade. Few larvae emerge, however, and of those that do few migrate up the plant, most are probably washed down the stem by rainwater. Even if some larvae do migrate to a healthy leaf, few invade. Thus, the adult by virtue of its high activity, is the chief agent of spread. Larvae are mainly confined to the interior of the leaf. Within brown sectors of the leaf the eelworms are subjected to desiccation during dry weather. Unlike the adults most larvae are killed by drying, only those in the green parts of the leaf, where high humidities are maintained, are able to survive. Thus, if the mesophyll cells brown quickly the newly hatched larvae may be killed by desiccation before becoming adults.

SUMMARY

The length of life-cycle of *Aphelenchoides ritzema-bosi* is 10 to 13 days. Each female lays about twenty-five to thirty-five eggs in compact groups. The eggs take 3 to 4 days to hatch and the larvae 9 to 10 days to reach maturity. Fertilisation and egg laying occur chiefly at the boundary between the discoloured and green portions of the leaf. The amount of free water inside the infested leaf is determined by weather conditions; eelworm activity in the leaf is, consequently, mainly confined to periods when there is rain or heavy mists. Early stages of chrysanthemum eelworm cannot resist desiccation as well as late stages. 95 per cent of late stage larvae and adults emerge from leaves immersed in water for 2 days compared with 12 per cent early stage larvae. Few small larvae invade leaves or migrate up the plant. Adults, unlike larvae, are able to swim because of the greater propulsive forces they generate. Adults and possibly fourth stage larvae are responsible for spread of infestations.

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SHORT COMMUNICATIONS

J. J. HESLING [1]): *Laboratory Note.* a. An alternative method of handling nematodes.
b. A technique for transferring delicate specimens to mountant.
c. Bubble "dissection" of immature *Heterodera* cysts.

a. GOODEY (1957) lists different instruments for handling nematodes. The slender barbed awns which are found on the seeds of many wild grasses are useful for picking up nematodes from some liquids. The awn is fixed with an adhesive (e.g. glyceel or paraffin wax) to the tip of a mounted dissecting needle with the barbs pointing towards the handle. When a nematode is lifted on the awn the barbs prevent it slipping off. The barbs do not damage the nematode which is easily floated off if it is "caught" near the awn-tip. Wild Barley (*Hordeum pratense* Huds.) provides a plentiful supply of suitable awns.

b. Great care is often needed when transferring delicate specimens from a clearing agent to the mountant on a glass slide; if they are handled by pipette or camel hair brush the liquid transferred with them may dilute the mountant. Specimens will usually adhere to a tiny drop of mountant on the tip of a fine glass rod and may be transferred in this way to the mountant on the slide. The amount of clearing agent carried over is very small and the specimen is undamaged. This method is useful when transferring the brittle cyst-cones of *Heterodera* spp. from xylol to canada balsam.

c. In cytological investigations on cysts of *Heterodera* spp. the oogonium, ovary, seminal receptacle and oviduct had to be mounted in one unbroken extended piece, but this was difficult because the organs intermingled in a gelatinous matrix. If the contents are gently pressed from a cut cyst into water on a glass slide and a drop of medium strength (40 vol.) hydrogen peroxide is added, this reacts with the gelatinous material and forces the eggs and organs apart. A hot needle passed over the slide bursts the bubbles so that the extent of the "dissection" can be seen. Unwanted eggs can be removed with a mounted eyelash leaving the reproductive organs clearly displayed.

GOODEY, J. B. (1957), *Laboratory methods for work with Plant and Soil Nematodes.* Tech. Bull. 2. Minist. Agric. London H.M.S.O. 3rd ed. 47 pp .

P. A. A. LOOF [2]): *Nomenclatorial note upon some* Pratylenchus *species.*

In his recent paper on the genus *Pratylenchus* the writer attributed the authorship of the combinations *P. brachyurus* (Godfrey, 1929) and *P. coffeae* (Zimmermann, 1898) to GOODEY (1951), and of *P. neglectus* (Rensch, 1924) and *P. penetrans* (Cobb, 1917) to CHITWOOD & OTEIFA (1952), as these authors were the first to use these four specific names in immediate juxtaposition with the generic name *Pratylenchus* and mentioned them as new combinations. Copenhagen Decision Nr. 105, however, which the writer had overlooked at that time, demands that "the author to be accepted as the author of that combination is whatever author first makes the transfer without qualification, it no being necessary for this purpose that he should actually cite the specific name in immediate juxtaposition with the name of the genus to which the species in question is so transferred". The transfer of these four species to *Pratylenchus* was made, although implicitly, by FILIPJEV (1936-a and 1936-b). He did not mention them, but evidently accepted T. Goodey's view that they were synonymous with *Tylenchus pratensis* De Man, 1880, which species was transferred to *Pratylenchus* in the papers mentioned. FILIPJEV & SCHUURMANS STEHKOVEN (1941) listed them as synonyms of *P. pratensis*

---

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[2]) Landbouwhogeschool, Wageningen, Netherlands.

(*neglectus* under the generic name *Aphelenchus*, the others under *Tylenchus*). As it is implicit in the wording of Copenhagen Decision Nr. 105 that the specific name must be mentioned somewhere in the paper by the author concerned, the authorship of these four combinations is to be attributed to FILIPJEV & SCHUURMANS STEKHOVEN (1941).

The author wishes to express his thanks to Miss M. Spillane, Londen, and to Dr. A. D. Baker, Ottawa, for kind advice.

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BUCHBESPRECHUNG

ZUR STRASSEN, O. *Neue Beiträge zur Entwicklungsmechanik der Nematoden. Zoologica* 38 (3. Lief.), Heft 107. Verlag E. Schweizerbartsche Verlagsbuchhandlung, Stuttgart 1959. Mit 14 Taf., 132 Abb., 132 Seiten. Preiss 132.— DM broschiert.

Die Nematoden waren seit jeher ein dankbares Objekt für entwicklungsmechanische Untersuchungen. Bekannte Forscher, wie BOVERI, SCHLEIP und GOLDSCHMIDT, haben hieran ihre Studien durchgeführt. Es ist erfreulich, dass nunmehr ZUR STRASSEN, der sich ebenfalls seit Jahrzehnten mit Entwicklungsfragen bei Nematoden befasst hat und bei seinen Untersuchungen über die Furchung des Pferdespulwurms (*Parascaris equorum*) zu teilweise anderen Vorstellungen gekommen ist wie BOVERI und SCHLEIP, hier versucht, diese Unstimmigkeiten aus dem Wege zu räumen, wobei er die „effektorischen" und die „determinatorischen" Entwicklungsgeschehnisse bei *Parascaris equorum* und *Bradynema rigidum,* einem kleinen Rundwurm aus der Leibeshöhle des Dungkäfers *Aphodius fimetarius,* untersucht. Dabei greift er jedoch sehr oft auch auf die Entwicklung von *Heterodera schachtii* zurück, die bekanntlich schon STRUBELL (1888) sehr sorgfältig studiert hat.

Der erste Teil, der der Beschreibung gewidmet ist, befasst sich mit der Bildung der Richtungskörper und den Furchungsstadien. Es wird u.a. darauf hingewiesen, dass der Hergang der Richtungskörperbildung bei *Bradynema* anders und bedeutend verwickelter ist als früher angenommen wurde. Auch die Entwicklung der Furchungsstadien hat manche Besonderheiten aufzuweisen. Die schon früher beobachtete „Invers"-Entwicklung bei *Parascaris* konnte bei einer Reihe weiterer Nematodenarten, wie *Bradynema, Rhabdias bufonis, Metastrongylus elongatus, Philometra sanguinea* u.a. beobachtet werden. Es liegt die Vermutung nahe, dass fakultative „Invers"-Entwicklung allen Nematoden eigentümlich ist.

Im analytischen Teil geht Verfasser auf die Gründe ein, welche die Nematodenentwicklung bedingen. Im Abschnitt „Effektorik" werden Bilateralität und Asymmetrie, im Abschnitt „Determination" Probleme der Selbstdifferenzierung, zytoplasmatische und nukleare Organisation sowie inverse Asymmetrie besprochen. Die Gesamtheit der im Zytoplasma vorhandenen qualitativ verschiedenen Determinatoren heisst Organisation. Als zur Zeit wahrscheinlichste Hypothese gilt, dass der Aufbau der Organisation hauptsächlich erst „mit der Konstituierung eines oder, bei Zwillingsriesen, zweier Furchungskerne beginnt und während der Furchung schrittweise vollendet wird".

Durch zahlreiche sehr instruktive Abbildungen wird dem Leser das Zurechtfinden in die oft verwickelten Vorgänge erleichtert. Ein sorgfältig ausgewählter Literaturnachweis beschliesst die Studie.

GOFFART (Münster)

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REPORT OF THE FIFTH INTERNATIONAL SYMPOSIUM ON PLANT NEMATOLOGY

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AUGUST 10-13, 1959

AT

UPPSALA

LEIDEN
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V International Nematology Symposium
Uppsala, August 10-13, 1959

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INTRODUCTORY LECTURE

BY

O. AHLBERG
Statens Växtskyddsanstalt, Solna, Sweden

Ladies and gentlemen,

As an introduction to this nematological symposium—the 5th international and the first in Sweden—it seems proper to give a short account of the history of plant nematology in Sweden.

At first, however, it is necessary to say a few words about the history and organization of the Swedish Plant Protection Institute, since this institute is the centre of Swedish investigations concerned not only with the control of injurious plant nematodes but also with their taxonomy, development and life-habits.

In 1875 the Royal Academy of Agriculture engaged a botanist, Jakob Eriksson, to study plant physiology and also, in some measure, plant diseases. Not until 1886 was a separate Institute of Plant Physiology established. However, in 1880 the Academy was able also to engage an entomologist and in 1897 an Institute of Entomology could start its work under the direction of Sven Lampa. Ten years later, in 1907, the two institutes were incorporated with the newly founded Central Institute of Agricultural Research as departments of botany and entomology and with Eriksson and Lampa as their directors. Lampa was in 1909 succeeded by Albert Tullgren and five years later, in 1914, Eriksson by Ernst Henning. In contrast to the botanists Eriksson and Henning, the zoologists Lampa and Tullgren never dealt closely with plant nematodes, because at that time these small and inconspicuous animals were looked upon as botanical matters, as they caused plant diseases.

In 1932 the Central Institute was reorganized, and out of its two departments for botany and entomology a new institute was built up, the present Plant Protection Institute, to which was added an advisory and plant quarantine department, this later being divided into two. There is now also a fifth department—for chemistry—and five branch stations in different parts of the country. At the foundation of the Plant Protection Institute the entomological department was named, more adequately, the zoological department but, naturally, entomology still dominates its activity.

As I said earlier it were the botanists who first took up plant nematological researches in our country. The first mention of a plant parasitic nematode in Swedish literature was in 1885, when Jakob Eriksson reported that he had found

at Pajala, high up in the north of Sweden, a gall-forming nematode in the roots of barley. He erroneously identified it as *Heterodera radicicola*, but soon afterwards it became obvious that it was identical with a nematode which at the same time Schøyen had described from Norway as *Tylenchus hordei* (i.e. *Ditylenchus radicicola*). Later it was stated that already in the sixties this species had done serious damages on barley not only in the north of the country but also at other places right down to Scania. It was also observed to attack meadow grass (*Poa pratensis*) and sea-lyme grass (*Elymus arenarius*). *Ditylenchus radicicola* is, thus, the first plant parasitic nematode to be with certainty discovered in Sweden. Nowadays it seems to have lost much of its former importance, probably since the highly susceptible two-rowed barley has been exchanged for other, more resistant varieties.

After 1885 there was an intermission of more than ten years before another nematode was discovered here. This species was *Heterodera avenae* or, as it was called at that time, *Heterodera schachtii*. It was found in 1897 on oats at Svalöv in Scania by the agriculturist A. G. Kellgren who emphasized, however, that attacks on beets had never been observed in the field.

In 1898 Ernst Henning published in the Journal of the Academy of Agriculture an exhaustive report on all plant parasitic nematodes which were then known in Sweden and elsewhere. Among Swedish species he mentioned *Tylenchus scandens* (i.e. *Anguina tritici*). Seed of wheat, evidently attacked by this nematode, had been observed in the province of Västergötland as early as in 1844 and some years later in other parts of the country too. Thanks to the modern methods of seed testing it is now of no real economic importance, since its occurrence is rare and limited to farms where the farmers use their own untested seed.

It is worth noting that Henning called attention to the fact that the stem eelworm, *Ditylenchus devastatrix* or, more correctly, *D. dipsaci,* had not yet been observed here and that no attacks had ever been observed on beets by *Heterodera schachtii*. Henning ends his report by expressing his complete conviction that the plant parasitic nematodes in Sweden were much more common than they seemed to be, judging from the scanty reports of their occurrence. He also says that a close and thorough investigation into the life-histories and economic importance of these pests must be considered as an investigation of great urgency.

The botanists, however, continued their nematological work, and the Swedish list of nematode pests was by and by enlarged. Our famous geneticist Herman Nilsson-Ehle published in 1903, in the Journal of the Swedish Seed Association, two papers in which he mentioned that not only the stem eelworm, *Ditylenchus dipsaci,* but also the true beet eelworm, *Heterodera schachtii,* had been found in Sweden. The former had proved to be the main cause of clover-sickness in many soils, the latter had been at last observed on beets. Like the zoologists at that time Ehle considered the cysts on oat and beets as belonging to the same species and said that *Heterodera schachtii* as a pest on oat put all other pests and diseases into the shade. Ehle was well aware of the potential danger nematodes are to the

cultivation of plants and warned expressively against them. He repeatedly returned to the nematode problem, the last time in 1920 in his important investigation "Über Resistenz gegen *Heterodera schachtii* bei gewissen Gerstensorten, ihre Vererbungsweise und Bedeutung für die Praxis".

Much later, in the thirties, *Heterodera galeopsidis* was discovered in the province of Östergötland by Ivar Wålstedt. Some years ago it was recorded also from the province of Hälsingland. It has, certainly, a much wider spread but seems, fortunately, to be quite harmless to economic crops, apparently attacking *Galeopsis* only.

As time went on, however, the zoologists seemed to realize that plant parasitic nematodes really belong to the animal kingdom and that, therefore, nematological problems are zoological problems mainly. The genetical problems in connection with the production of nematode resistant plant varieties are still in the botanists' hands, since there are not many zoologists in Sweden dealing with genetics.

The earliest zoological contributions to plant nematology consisted of very short notes appearing in the pest-reports published by the entomological department of the Central Institute. The first of these reports came in 1911 and was, at irregular intervals, followed by six more, the last one in 1934. There was, however, not more than one new species mentioned in them: *Aphelenchoides fragariae* which was observed in 1911 at Sigtuna where 2000 strawberry plants were said to have been fatally damaged.

In 1927 the first nematological investigation—the first study which really deserves to be called an investigation—was taken up by N. A. Kemner who was then assistant professor of the department. He took up studies on *Heterodera rostochiensis, H. schachtii* and *Ditylenchus radicicola*. His papers on these three species were published in 1929 and 1930. After he was appointed a professor at the University of Lund his investigations on the potato root eelworm were taken over by me.

Without any doubt potato root eelworm is one of the most serious pests on cultivated plants in Sweden and the sole nematode species the control of which is subject to legislation. It is very widely spread all over Götaland and Svealand, i.e. in all provinces south of the river Dalälven, and, further, in a few isolated areas in the provinces of Gästrikland and Hälsingland. In our other northern provinces it has not as yet been observed. Our main problem is, then, how to prevent its further spread to the north, where the potato-growing area must be enlarged in order to establish a better and more varied crop rotation, which, for several reasons, is sorely needed. Such increase in potato growing for which the seed potatoes will have to come partly from the infested south, will, of course, increase the difficulties in preventing the spread of the potato root eelworm.

Our investigations have shown that this eelworm must have come to Sweden many years before it was at first discovered here in 1922. In 1933 I found in the province of Värmland, near Karlstad, a little plot where potatoes had once been grown. Yields had decreased yearly and, in spite of good cultivation and

manuring, finally failed at the beginning of the first world war or earlier. In the examination of the soil samples, taken from this plot, I found many empty cysts of the potato root eelworm. There is, then, no doubt that this eelworm had been the cause of the crop failure in that plot and that it had existed there at least since the first years of the century. In view of the isolated situation of the little plot and its being cultivated for household use only, we must assume that the eelworm was brought there from some potato-plot not too far away and that the eelworm must have existed in the province at least since the beginning of the nineties. Considering that the initial spread of the nematode population is fairly slow and that potato-growing in Sweden at that time had not reached the same intensity as it has to-day and, further, that only intensive potato-growing year by year in the same soil makes it possible for the eelworm to become a serious pest, we may assume that the potato root eelworm has existed in Sweden at least since the middle of the last century.

Another cyst-forming nematode which, lately, has begun again to be a serious pest in some of our southern provinces is the oat nematode, *Heterodera avenae*, and within our sugar-beet-growing districts we have, of course, to reckon with damage by the beet nematode too, but thanks to the careful and effective planning of crop rotation and other measures taken by the Swedish Sugar Manufacturing Company the losses are held within tolerable limits.

A nematode problem of vital importance within our strawberry-growing districts is the control of the nematode parasites of the strawberry plant. Among them *Ditylenchus dipsaci* and *Pratylenchus pratensis* seem to be quite as dangerous as, or perhaps more dangerous than, *Aphelenchoides fragariae* which has until now been considered with us as the only strawberry nematode. These three nematodes began to cause such heavy losses that the growers were considering giving up strawberries altogether. Justly convinced of the beneficial effect of good crop rotation some growers then tried to use a rotation with three years fallowland between each strawberry crop. These measures proved to be very beneficial but, unfortunately, also very costly. The growers, therefore, have now, following a Danish method, started to use warmwater treatment of the young plants and to grow them in non-infested soil tested by our zoological department. In this way we seem to have solved in an economically acceptable way the problem of the control of these pests.

Other problems of ours depend upon our considerable importation of living plants, flower bulbs, seeds and so on, which exposes us to the danger of getting many nematodes, still unknown to us, established and spread here. Infested greenhouse plants can easily cause infestation of the soil of the open fields, because gardeners renew the soil of their houses at more or less regular intervals usually spreading the old soil in the fields without any desinfection or other precautionary measures.

I do not intend at this time to give a complete list of our nematode problems. They do not, I think, differ much from the problems in other countries. The

most serious attacks which have given cause to considerable economic losses for many farmers or market gardeners have, however, been useful in so far as they have drawn public attention to these problems and made people realize, to some extent at least, the enormous importance of these small pests. This fact, I hope, will lead to the provision of nematological facilities at the Plant Protection Institute corresponding to the importance of these pests, and the establishment of sufficient staff who can devote their entire attention to nematological work.

I cannot, however, omit telling you on this occasion that we, who work on nematodes at the Plant Protection Institute, are profoundly grateful for the truly valuable example and counsel we have received from our much more learned colleagues abroad, especially in England, Germany and the Netherlands—and all the others who must not be forgotten. I take this opportunity to thank you all. It is my hope—an audacious hope, perhaps—that we shall be able some time in the future to pay in some way or another some part of our debt.

Before ending this account of Swedish plant nematology I wish to say some words also about recent work going on in Sweden to improve genetically quite a lot of our cultivated plants and to produce more and more nematode-resistant varieties. First I want to mention the Swedish Seed Association, especially its branch station here at Ultuna where our friend Bingefors is working mainly on the improvement of clover varieties. He is, however, not the first of the scientists of the Association to work on these clover problems. The most prominent of his predecessors is Nils Sylvén who gave us some 30 years ago the clover-strain Merkur with its fairly good resistance against stem eelworm. Among the scientists of the Association I also mention Ivar Wålstedt, who still continues his work on nematode resistant cereals.

Other institutes working on nematode resistance in cultivated plants are the State Horticultural Research Station at Alnarp and the Balsgård Fruit Breeding Institute at Fjälkestad — both in Scania. Both institutes have been for some years working on nematode resistant strawberry plants.

I have already said that I do not intend to give more than a short account of plant nematology in Sweden. I think, therefore, that it is now high time to stop before this account becomes a long one and to express my hope that this symposium will be successful and that it will be followed by many other symposia in a spirit of concord and good fellowship.

Nematologica (Suppl. II, 1960): 6-12. — E. J. Brill, Leiden.

SAFETY IN SOIL TREATMENTS FOR EELWORM CONTROL

BY

JOHN GRAINGER

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The first successful attempts to control eelworm diseases, apart from the prohibitively expensive practice of steaming the soil, began little over a decade ago. There are now four general types of treatment:

1. The relatively traditional method of injecting volatile liquids at intervals through the soil mass.
2. Drenching the soil with water containing 'solubilized' chemicals of the phenol-xylenol series (STANILAND & STONE 1952).
3. Surface treatment of the soil for surface-feeding eelworms (GRAINGER 1956).
4. The intimate mixing of non-volatile materials with the soil mass (GRAINGER 1958).

Soil drenches are, because of their large water requirements and relatively high cost, most suitable for glasshouse treatment. The other methods are, however, applicable on a full field scale and the need for such extensive treatment increases with our growing awareness of the serious effects of many eelworm diseases. Volatile materials may be dangerous to humans, as chloropicrin, or at least objectionable like D.D. Intimate mixing of materials with soil has so far been most successful with a dust containing yellow oxide of mercury (YOM)—a poisonous substance—and other materials under test are not much less deleterious. Surface treatments have been given with a dilute solution of mercuric chloride. It seems desirable to make all these operations of treatment as safe as possible, for it is inevitable that they will be used increasingly in the future.

INJECTION

Most experimental work and much normal glasshouse treatment has been done with hand injector guns, and for safety it is only desirable to fill the container from a drum provided with a tap and air vent. When injection has to be done on a field scale, safety precautions are (a) the liquid should be pumped from its delivery drums into the injector tanks, or the delivery drums themselves should be used as tanks on the machine, and (b) a system of injection with no pressure build-up should be chosen.

A suitable tank capacity for a field injector is 40 gall. (about 180 litres). Pumps

J. GRAINGER: *Safety in soil treatments for eelworm control.*

Fig. 1. A pump (Allman G. 45 Rollervane) suitable for filling field-scale injector tanks. It can deliver 3,300 gallons per hour at 545 r.p.m., the speed of a tractor power take-off.

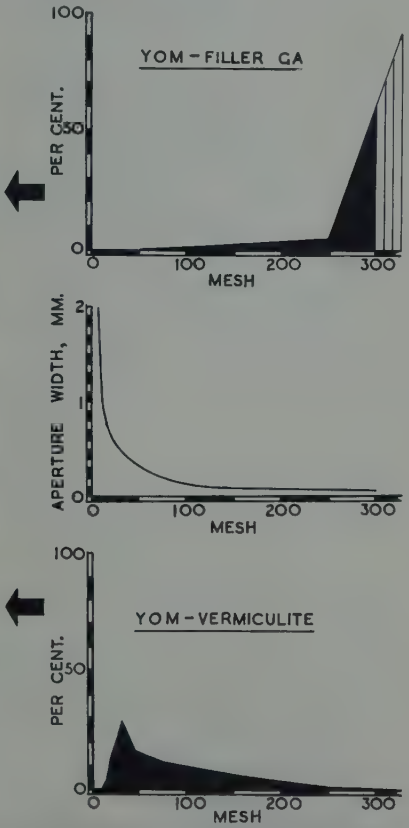

Fig. 4. Particle size ranges of dust fillers and safety of machine operation. Both photographs were taken on the same day and under the same relatively dry condition of the soil, when the safe vermiculite formulation showed no dust cloud whatever.

are now available to work off the relatively slow speed (545 r.p.m.) of a tractor power take-off; that shown in Fig. 1 is capable of delivering 3,300 gallons (150,000 litres) per hour, and would fill a 40 gall. tank in less than ¾ min. of actual pumping. This represents a very considerable saving of time over hand

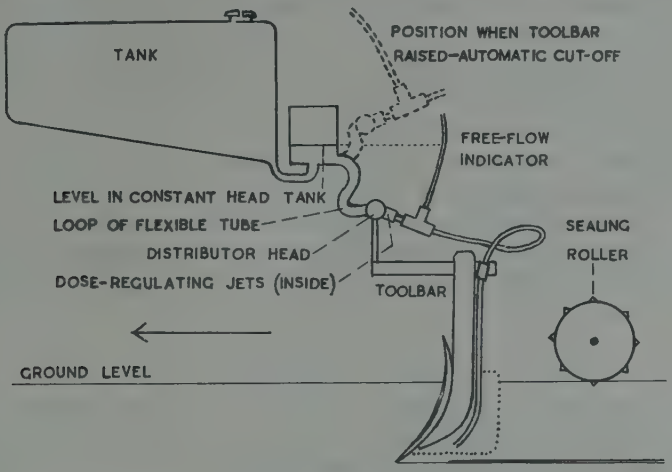

Fig. 2. A system for safe gravity injection (GRAINGER 1951) with automatic flow control when the toolbar is lifted or lowered. An important new safety feature is that the free-flow indicator tubes of transparent polythene should be long enough to rise above the level in the float-controlled constant head tank when the machine is in the working position.

filling, which, even under the best organization, takes something like 15 min. Centrifugal or gear pumps could be used, but would require a speed greater than that of a tractor power take-off.

The 'Auchincruive' system of gravity injection (GRAINGER, 1951) was designed

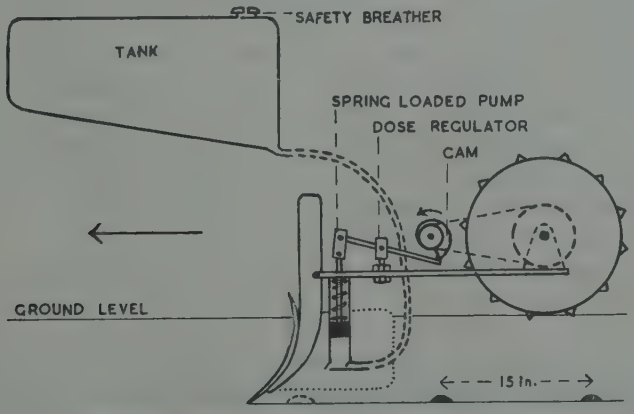

Fig. 3. A system for safe interrupted flow injection.

for safe, pressure-less injection, but if free-flow indicator tubes are used, they should be made long enough to extend above the level of the constant head (Fig. 2). If any blockage occurs, the liquid rises in the tube but does not overflow. The 'Auchincruive' system delivers the liquid in continuous traces, but there are indications that 'spot' injections give superior performance on some soils. A system like that shown in Fig. 3 is then quite safe, though a diaphragm pump is more free from trouble in practice than the plunger pump suggested by the diagram.

The combination of tank filling by pump with pressure-less injection ensures that the liquid injected shall never come in contact with the operator, and will not break out in dangerous jets if the system should leak. Other useful items are the provision of safety breathers (GRAINGER, 1951) on the tanks, frequent strainers in the system, and the use of galvanised iron, brass or copper for the metal parts of injectors. Blockages are thereby reduced to a minimum.

SOIL DRENCHING

Soil injection with D.D. combined with a soil drench containing solubilised xylenol has given very high degrees of control (97 %) of eelworm diseases in glasshouses or small areas where the water requirements can be met, and the relatively heavy cost justified by high crop value. Solubilised xylenol is diluted 1 to 100 with water, and then hosed on to the soil either by gravity or by pump, a coarse rose being used for gentle distribution. The undiluted material damages human skin, and even the dilute solution is not good for sensitive skins, so rubber gloves should be worn by operators.

SURFACE MIXING

This method was devised (GRAINGER, 1956) for the treatment of the tulip-root eelworm, *Ditylenchus dipsaci,* on a low-value crop (oats). It is of limited application, for a highly resistant though only medium-yielding oat (Milford S225) is available. The usual safety precautions are necessary in the storage of the mercuric chloride used to prepare the 1 in 1,000 solution, but pumping the liquid in the tanks would again be desirable. It is, however, likely that surface treatment, if and when required, will be replaced by a shallow depth treatment along the lines mentioned in the next section, particularly as tulip root frequently occurs along with the cereal eelworm *Heterodera avenae.*

MIXING NON-VOLATILE MATERIALS

A method of evenly incorporating mercury-containing dusts and other non-volatile materials into the cultivated depth of soil has recently been developed into a highly economic field scale treatment by means of a new machine (GRAINGER,

1958). Twelve bushels of dust containing 5 lb. Hg equivalent as yellow oxide of mercury (YOM) per acre (925 litres containing 5.6 Kg per hectare) was originally the standard dose, but machine mixing was found effective with about half these amounts. The dust is placed by the machine in two equal layers, one on the soil surface and the other at 7 in. (18 cm.) depth in the soil; even mixing to a depth of 9 in. (22 cm.) is then accomplished by means of a rotary cultivator (rotavator) working to that depth. The new method has brought increases in main-crop potato yields between 1.4 and 3.5 tons per acre over control yields of 3.2 to 7.7 tons per acre respectively (GRAINGER, 1959), according to the degree of infestation by *Heterodera rostochiensis*. Eelworm populations were reduced by an average of 78 %, compared with about 55 % by D.D. under similar conditions and certain fungal and bacterial soil pathogens were also controlled. An effective dose of formulated dust would cost something like £ 7 to £ 10 per acre, making it the cheapest soil-mass treatment known, and conferring a ratio of cost to benefit of up to 1 : 5.8 under suitable conditions. The relatively high field percentage of eelworm control makes it possible to envisage almost complete control from three consecutive treatments preferably spaced by usual rotations. So many advantages would, however, be worthless if the methods were not safe in practice, and the following factors have been used to make it so. (i) The use of very small doses of mercury compound per acre. (ii) Arranging the time of treatment so that any mercury compound is reduced to elemental Hg in the soil before the host crop is planted. Given this precaution, even a poisonous compound of mercury can be used if the machine operation is designed to permit no escape of diluted dust into the air. This has been achieved by (iii) the use of a very light but coarse and dustless carrier for the mercury compound. It not only eliminates any escape during the mixing operation, but also (iv) gives complete safety in filling the hopper. (v) Finally, the machine itself provides an enclosed path for the dust. The following paragraphs amplify these points.

(i) Recent results from field control trials show that machine mixing of as little as 2½ lb. Hg per acre (2.8 Kg per hectare) is quite as effective in controlling potato root eelworm as treatment at double that dose. This small amount of mercury compares favourably with about 8 lb. Hg per acre (9 Kg per hectare) already in extensive horticultural use for transplant control of club root disease (*Plasmodiophora*) of brassica crops. The poisonous mercuric chloride is sometimes used for the transplant treatment without ill effect on the operator or consumer.

(ii) Mercuric oxide or yellow oxide of mercury (YOM) is produced in commerce as a fine-particle compound, and, though poisonous, was found to be much superior for soil disease control to the non-poisonous mercurous chloride which has relatively large particles. BOOER (1944) has, however, shown that both these compounds (and most other compounds of mercury) are reduced to elemental Hg in normal soils and so presumably eliminated from uptake by the host plant. Once the mercury compound has been reduced to elemental Hg there would seem to be no subsequent human hazard. The writer, his staff and family have all

eaten potatoes grown on ground treated at the full rate of mercury, with no ill effect whatever. ROBSON (1919) states that tomatoes from plants grown on ground treated with 2½ cwt. and 7¾ cwt. per acre of mercuric chloride as a water drench were eaten and no bad results were reported. There has, so far as the writer is aware, never been the slightest adverse gastronomic effect on the consumer from the mercury transplant treatments mentioned above; they would not be used so extensively if there had.

The reduction to metallic Hg seems to take about two days under Auchincruive conditions, but we originally specified at least six days between treatment and planting, to provide a safe minimum period. More recent results show, however, that disease-control performance is enhanced by an even longer interval, and treatment 33 days before planting (the longest time yet tried) has so far given better performance than any shorter period. This is probably because Hg vapour has then had time to diffuse well through the soil by the time larvae of the parasite have emerged from their cysts under the stimulus of root exudate from the host.

(iii, iv and v) Since the reduction to elemental Hg in the soil removes any subsequent danger, it remained to make the initial mixing operation safe, to design the machine and to formulate the dust so that the material did not escape into the air and was never handled by the operator. The use of a dustless carrier, arriving at the farm in sealed, lightweight packages with no more than 0.5 % by volume of even a poisonous compound of mercury, could not in an enclosed machine be even slightly dangerous.

It was thought originally that a fine-particle carrier would be necessary to dilute the fine-particle YOM, and most development work was done with such a material—filler G.A., a diatomaceous material of high volume weight (77½ lb. per cubic foot) with 93 % of the particles passing a 300-mesh sieve (fig. 4, top right). A special dusting arrangement was found necessary to deliver such a heavy dust in the machine. It was picked up from the base of the covered conical hopper (Fig. 4) by the diesel exhaust stream from the tractor engine and then fed into a booster fan through an enclosed turbulence chamber which mixed the dust evenly with the exhaust stream. From the fan the dust was divided into equal streams, half going to a series of surface deflectors and the other half delivered to the spaces behind duck-foot, cultivator tines for the application in depth. The dust was therefore delivered in a totally-enclosed system except for the surface deflectors. These, however, were placed so close in front of the rotavator that as soon as the dust reached the soil surface it was immediately incorporated. The machine itself was thereby made safe.

This arrangement permitted no dust to escape when the ground treated was moist, but under dry conditions a cloud arose from the treated soil and not from the machine itself (Fig. 4, top left). This problem was solved by diluting the YOM with a coarser carrier—the 'fine' or X1 grade of vermiculite. This has an apparent density of only 9 lb. per cubic foot before mixing with YOM, and its

particle size varies over a wide range, with the larger granules predominating (fig. 4, bottom right). It is very friable under all conditions and flows freely in the machine. Though it costs about four times as much by weight as G.A., only about a tenth the weight is needed to give the requisite volume per acre, so that a considerable saving in the cost of diluent is effected. Performance in controlling eelworm is as good as with the heavier dust and, most important of all, there is absolutely no dust escape from the machine or soil during application, even under the driest British conditions (Fig. 4, bottom left). The YOM vermiculite dust as compounded has a volume weight of about 14 lb. per cubic foot, and even a large bag of it can be lifted easily. The hopper can therefore be filled with safety from light packages as they arrive at the farm, without touching the dust by hand, or sending a cloud from the hopper, as frequently happened with the more awkward operation with the heavier and dustier carrier.

A third and bad type of dust was found during the investigation—the 'clinging' or tenacious carrier. This property was generally found among the finer materials, but was not necessarily related to particle size. A 'clinging' dust adheres to metal surfaces, thus at least hindering free flow in the machine if it does not actually block the smaller delivery pipes. If a clinging dust is also hygroscopic, this difficulty becomes a catastrophe. Tenacious dusts, moreover, are generally poor in control performance. Recent results of pot experiments show that the vermiculite formulation of YOM gave 73 % reduction of eelworm attack on potato roots, with 24 % increase in tuber yield over a growth cycle of 130 days. This is quite equal to the performance of equal amounts of YOM compounded with the non-clinging filler GA, but equal doses YOM mixed with a clinging sample of Kieselguhr gave, under the same conditions, only 46 % control of eelworm attack with no increase in tuber yield, and field results also agree. A clinging dust probably 'blankets' the YOM particles, delays their normal reduction to elemental Hg, and probably also postpones the subsequent diffusion of mercury vapour through the soil. The vermiculite formulation, on the other hand, is the least tenacious and most friable material so far investigated.

It is probable that future field control of eelworm diseases will lie more with soil mixing than with other methods, for it already gives by far the least expensive and most efficient field treatment when used with mercury. The use of a new dustless formulation as specified, in the machine as designed, makes the operation of mixing completely safe, whilst reduction to elemental Hg in the soil removes any subsequent ill effect. Other materials might well be tried now that the machine is available, but whether the other materials are themselves poisonous or not, the provision of 'dustless' formulations and a safe machine are very important in practice.

All the dust formulations used in this investigation have been compounded by Messrs. F. W. Berk and Co., London, to whom the writer tenders thanks for valued collaboration.

ZUSAMMENFASSUNG

Sicherheit in Bodenbehandlung zur Bekämpfung der Nematoden.

Die meisten Stoffe, die für eine Bodenbehandlung gegen Nematoden verwendet werden, sind für Menschen unangenehm, wenn nicht giftig. Die Geräte müssen darum so konstruiert werden, dass sie grösste Sicherheit beim Handhaben gestatten. Bei Feldarbeiten mit Injektoren oder bei Flächenbehandlung ist es notwendig, den Stoff vom Fass zum Behälter zu pumpen, oder das Fass selbst als Behälter auf dem Gerät zu verwenden. Ein druckloses Injektorsystem und eine zuverlässige Kontrolleinrichtung für den gleichmässigen Durchfluss der Flüssigkeit sind dabei erforderlich. Eine neue Methode für den Zusatz von gelbem Hg-Oxyd oder anderer nicht flüchtiger Stoffe bis zur Pflugtiefe ist ungefährlich bei Verwendung sehr kleiner Mengen (2.8 bis 5.6 kg je Hektar) in Verbindung mit einem Träger (Vermiculit) von geringem spezifischen Gewicht, der in Behältern verpackt ist, die leicht zu handhaben und genügend stark sind; ferner durch Verwendung richtiger Geräte, um während der Arbeit eine Staubentwicklung völlig zu vermeiden. Die meisten Hg-Verbindungen werden in fast allen Böden zu elementarem Hg reduziert, sodass schädliche Folgen vermieden werden. Die Mengen von Hg sind geringer als die Mengen von 9 kg je Hektar, die schon im grossen Umfang ohne schädliche Wirkung für die Bekämpfung von Kohlhernie gebraucht worden sind.

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CONTROL OF PLANT PARASITIC NEMATODES

3. THE FINAL EELWORM DENSITY IN SOIL AS A MEANS OF ASSESSING THE EFFICACY OF A FUMIGANT

BY

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To estimate the effect of fumigants on the potato root eelworm, *Heterodera rostochiensis* Woll., accurate methods of determining both the concentration of fumigant at the site of action and the viability of treated eelworms are necessary. Concentrations of ethylene dibromide in soil (CALL, 1957) and methyl bromide in chambers (LUBATTI & BLACKITH, 1956) have been determined by catalytic decomposition. For present experiments methyl bromide was chosen in preference to ethylene dibromide as the distribution in fumigation chambers was known and nematodes could therefore be fumigated at known dosages.

The problem of estimating kill is more difficult. The viability of encysted potato root eelworms has, in the past, usually been estimated by hatching the treated larvae in potato root diffusate (PETERS & FENWICK, 1949; PETERS, 1952). The root diffusate assay has however been shown to give rather variable results (FENWICK, 1957; HAGUE, 1959a), the kill rising as the time between fumigation and assay increased. The final population after injection following a potato crop (PETERS, 1952) was therefore investigated to see whether it could be adapted to dosage-response experiments.

HAGUE & HESLING (1958) and PETERS (reported by FENWICK, 1955) found that the final density in terms of "eggs per gramme of soil" increased to a maximum and then declined when potatoes were exposed to a geometric series of inoculum densities, giving a hump-shaped curve which demonstrates that relatively low initial densities may give rise to final ones greater than those produced by higher initial densities. In nematicidal experiments it is clearly essential to work on the rising phase of this curve so that the final densities in all treatments shall be less than in the controls or else ambiguous results may be obtained as in PETERS' (1952) experiments, where the use of high initial densities on the falling phase of the curve resulted in higher final densities in some treatments than in the controls. It is also important that there should be no apparent reduction in size and yield of the host plant otherwise the final eelworm density may be reduced. From a series of experiments it has been found that an initial density of the order of

50 eggs per gramme of soil satisfies the above requirements for pot work.

The present paper describes some experiments in which air-dry cysts (free from soil) and soil (artificially infested with a known number of cysts) were fumigated with methyl bromide in closed steel chambers. In the first experiment, a root diffusate assay is compared with two forms of the final density assay.

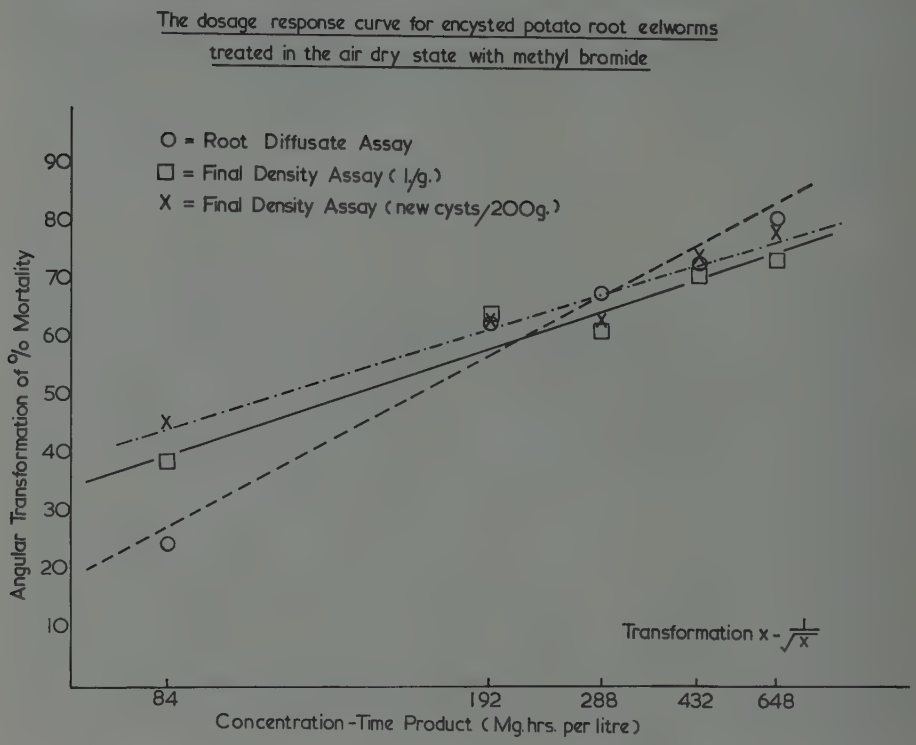

Fig. 1. The dosage-response curves for encysted potato root eelworms treated in the air-dry state with methyl bromide: three forms of assay compared.

Experiment 1. Treatment of air-dry soil-free cysts.

Batches of 1000 cysts (for final density assay) containing 396 eggs per cyst and batches of 50 cysts (for root diffusate assay) containing 640 eggs per cyst were fumigated in solid watch glasses, in a steel chamber of 345 litres capacity with methyl bromide. The introduction of the fumigant into the chamber and the determination of the concentration of methyl bromide in the chamber were carried out as described by LUBATTI & BLACKITH (1956). The cysts were exposed to methyl bromide at a range of concentrations for eight hours, replication being two-fold for the final density assay and three-fold for the root diffusate assay. On sampling no significant loss of methyl bromide could be detected so the effective concentration was the same as that introduced into the chamber.

Root diffusate Assay

After completion of fumigation the cysts were soaked in water for one week and then a three week hatching test was carried out. The cumulative hatch was counted from which the percentage mortality was calculated by the following equation:

$$\% \text{ Mortality} = \frac{(L)c - (L)t}{(L)c} \times 100$$

where $(L)c$ = mean hatch from 50 untreated cysts
$(L)t$ = mean hatch from 50 treated cysts

Final Density Assay

After fumigation each batch of 1000 cysts was mixed with 7 kg of uninfested soil (= 6.35 kg of air-dried soil); the initial density in the controls was therefore 62 eggs/g of air-dried soil. Each batch of infested soil was placed in a clay pot (internal diameter 10 in.), plunged in a sand-gravel mixture and an Arran Banner potato planted in each pot. After the death of the potato haulms, the soil was air-dried and soil samples each of 200 g were washed in the Fenwick can to estimate the cyst density in the soil.

The following equations were used to estimate percentage mortality:

1. *New cysts per 200 grammes of soil*

$$\text{Mortality} = \frac{[(c/200\,g)c - (c/200\,g)a] - [(c/200\,g)t - (c/200\,g)a]}{(c/200\,g)c - (c/200\,g)a}$$

$$\% \text{ Mortality} = \frac{(c/200\,g)c - (c/200\,g)t}{(c/200\,g)c - (c/200\,g)a} \times 100$$

where $(c/200\,g)c$ = the final cyst density per 200 g in the controls
$(c/200\,g)t$ = the final cyst density per 200 g in the treatments
$(c/200\,g)a$ = the initial cyst density per 200 g

2. *Eggs per gramme of soil* (= c/g x 1/c). The estimations of eggs per cyst were obtained by using the homogeniser technique of Bijloo (1954).

$$\% \text{ Mortality} = \frac{(1/g)c - (1/g)t}{(1/g)c} \times 100$$

where $(1/g)c$ = the mean final density in the controls
$(1/g)t$ = the mean final density in each treatment

Results

The results are shown in Table I where the means of the percentage mortalities have been calculated for the three forms of assay.

TABLE I

The effect of fumigating potato root eelworms with methyl bromide assayed by three methods

		Concentration-Time Products. mg.h/l					
		84	192	288	432	648	Control
Root Diffusate Assay	Mean Hatch of larvae per 50 cysts	13425	3197	2297	1377	369	16275
	% Mortality	18	80	86	92	98	
Final Density Assay	New cysts per 200 g	339	143	140	48	28	700
	% Mortality	52	80	80	93	96	
	Eggs/g	572	196	222	97	75	947
	% Mortality	40	79	77	90	92	

In this experiment a sufficient number of dosage levels were chosen to apply the method of regression analysis. The percentage mortalities were transformed to the angular scale and plotted against the reciprocal root of the concentration-time product (Fig. 1).

No significant difference was found between the regression lines for the two forms of the final density assay but the regression line for the root diffusate assay differed significantly ($P = 0.5$) in slope from that of both forms of the final density assay. It is however probably inappropriate to compare the root diffusate assay with final density assays in this way as assay technique (hatching and cyst counts or homogenising) is confounded with time of sampling (i.e. immediately after fumigation for the root diffusate assay and the end of the growing season for the final density assay).

The final density assay (new cysts per 200 g) gives a value of about 90 mg.hrs per litre for the median lethal dose (L.D. 50) for methyl bromide. By extrapolation it is clear that a concentration-time product in excess of 648 mg.hrs per litre would be required to give an effective kill of encysted larvae in air-dry soil-free cysts, thereby confirming earlier work by FRANKLIN (reported by LUBATTI & BLACKITH, 1950) and by HAGUE (1959a).

Experiment 2. Treatment of artificially infested soil

Artificially infested soil was fumigated with methyl bromide in large pots (10 in. in diameter holding about 7 kg of moist soil) in a steel chamber of about 5500 litres capacity. The range of concentration-time products did not exceed

200 mg.hrs per litre at which dose moistened cysts are known to be effectively killed (HAGUE 1959b). Replication was three-fold.

A preliminary experiment had shown that methyl bromide penetrated rapidly into pots of moist soil if the moisture content of the soil did not exceed Field Capacity. The concentration in the soil after two hours was found to be in equilibrium with that in the air-space of the chamber, and therefore the amount of methyl bromide introduced into the chamber was taken as the effective dose.

The experimental details are shown in Table II. The cysts were conditioned

TABLE II

Treatment of infested soil

Cyst Inoculum per pot	1000 c/pot
Viable content of cysts (eggs/cyst)	415 e/c
Weight of air-dried soil per pot	6.39 Kg
Initial Infestation (cysts/200 g)	31.3 c/200 g = (64.9 e/g)
Moisture Content of soil when fumigated	10.4 %
„ „ „ „ at Field Capacity	22.2 %
Concentration-time products (mg.hrs per litre). 100 : 120 : 140 : 160 : Zero.	
Time of exposure to fumigant	20 hrs

in soil for one month and, after fumigation, the same procedure was followed as in the previous experiment except that height of haulm and weight of tubers were recorded. Final cyst densities were obtained by the usual methods and the percentage mortalities calculated in terms of new cysts per 200 g.

Results

Table III shows the height of haulms, yield of tubers, number of new cysts per 200 g of soil and the percentage mortalities calculated in terms of new cysts per 200 g. Height of haulms and weight of tubers, shown by PETERS (1948) to

TABLE III

Fumigation of infested soil with methyl bromide
Means of three replicates

Concentration -time product mg.h/l	Height of haulms cms	Weight of Tubers in grammes	New Cysts /200 g	% Mortality
100	79	310	252	51
120	79	427	53	90
140	72	392	12	98
160	78	398	3	99
Control	77	393	517	

be fairly sensitive as criteria of plant growth, gave no significant differences between treatments and controls; thus there is experimental evidence to show that one of the prerequisities for final density assays is satisfied, i.e. that there should be no effect on the plant.

The criterion new cysts per 200 g shows clear treatment differences (see also Table III). A linear relationship was shown to exist (see Fig. 2, regression line for infested soil) between the angular transformation of the percentage mortality and the concentration-time product (as a reciprocal transformation). The L.D. 50 of 100 mg.hrs per litre is of the same order as that obtained in the fumigation of air-dry cysts (90 mg.hrs per litre) but the slope of the regression line for infested soil (Fig. 2) is much steeper. Clearly cysts in soil are much more susceptible to methyl bromide fumigation than air-dry cysts.

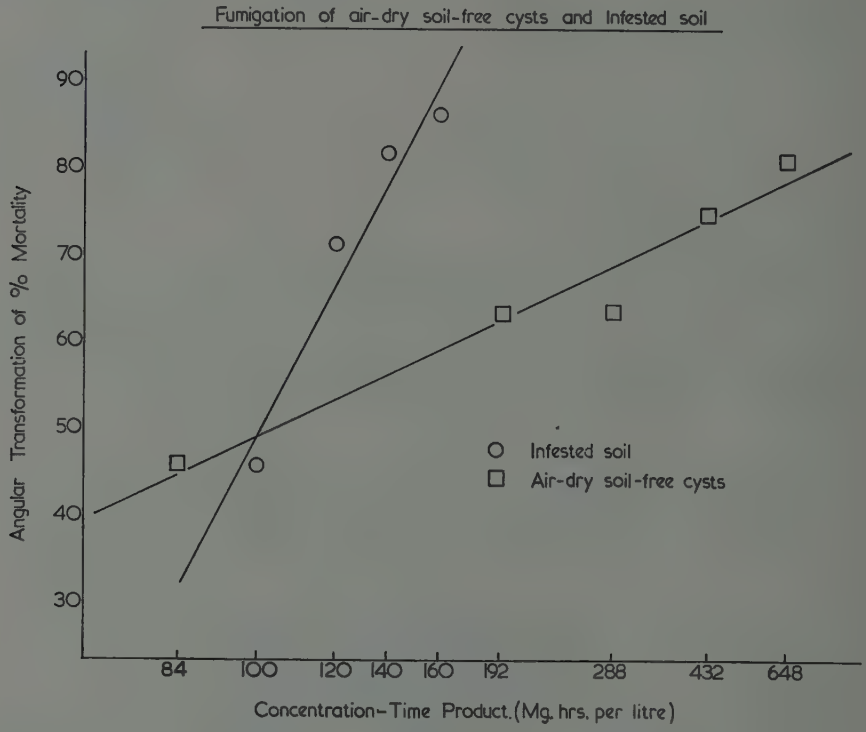

Fig. 2. Comparison of dosage-response curves for the fumigation of air-dry soil-free cysts and cysts in soil with methyl bromide.

Discussion

Satisfactory dosage-response regression lines have been obtained using final density assays. Hence it is possible to relate the nematode response to a given

dosage of nematicide and thus to compare the potency of various chemicals in the same way as for insecticides. Also cysts in different states can be compared using a standard fumigant as in the present experiments, i.e. air-dry cysts and cysts in soil (Fig. 2). Methyl bromide is extremely useful for experimental work as its concentration can be determined accurately and the fumigation ended abruptly (e.g. by opening the door of the fumigation chamber) thus enabling narrow dosage ranges to be used. In soil it is difficult to control the conditions of fumigation because the nematodes are of necessity exposed to treatment for long periods and it is thus difficult to get precise estimates of the dose applied. However recent work (CALL & HAGUE, unpublished) has shown that a similar dosage-response relationship can be obtained for ethylene dibromide, the L.D. 50 being of the same order as that for methyl bromide, i.e. 100 mg.hrs/litre.

In fumigation experiments the exposure of cysts to a graded series of doses should result in a corresponding reduction in the viability of the cyst contents. In the presence of the host plant these viable remnants should behave in the same way as a graded series of inocula in population experiments (see HAGUE & HESLING, 1958): the results of the present experiments support this view. Eelworm populations resulting from fumigations will thus multiply at different rates and therefore the final density assay is related to the true mortality, in the initial infestation, by the multiplication rate. If an estimate of the kill in the original infestation is required, it necessitates measuring the multiplication rate at varying initial densities of UNTREATED cysts in order to back-calculate the kill in the original infestation: final densities could then be used to estimate initial densities which are themselves the results of treatments. It seems unnecessary in nematicidal experiments to carry out parallel trials with untreated cysts as long as the original infestation does not exceed that which gives rise to the maximum final density: all treatments should then give rise to final densities less than the maximum.

The final density assay is clearly dependent on two factors, the host plant and the soil, and these may confound the result in many ways. However the final density assay is an improvement on the root diffusate assay as it seems unlikely that it would be affected by a delayed response. The production of new cysts is the consequence of successful root penetration by the infective second stage larvae and it may well be that a larval invasion assay could be carried out in a shorter time than the final density assay.

What are the errors associated with final density assays? Where "eggs per gramme" are estimated, microscopic examination of cyst contents (opened by the homogeniser technique) may not distinguish between living and dead eggs. Non-viable eggs are likely to be counted as viable and thus low estimates of kill are obtained particularly at high dosage levels. Where "new cysts per 200 g" is used as the criterion, the assay does not take into account the viable remnant of eggs in the original cyst population.

There was no significant difference between regression lines obtained from the criteria "eggs per gramme" and "new cysts per 200 g" of the final density

assay. On this evidence the criterion "new cysts per 200 g" is to be preferred to "eggs per gramme" as it eliminates the estimate of "larvae per cyst" thus requiring less labour. The final density assay (new cysts per 200 g) is therefore a simple procedure of estimating the cyst content of the soil before fumigation and after growing the host plant in the treated soil. "New cysts per 200 g" is a purely arbitrary figure and the unit of soil could be adjusted to suit sampling requirements, i.e. per gramme, per 100 g, per 100 cc etc.

Can this form of assay be used for all species of *Heterodera*? The method appears suitable for those species which have only one complete generation per year such as *H. rostochiensis, H. avenae* and *H. göttingiana.* For *H. schachtii, H. cruciferae* and similar species which have several generations per year, treatment differences could well be obscured by rapid multiplication. This could to some extent be overcome by stopping the experiment at the point when one generation had been completed. The final density assay seems therefore to be applicable, unlike the root diffusate assay, to all species of *Heterodera.*

ZUSAMMENFASSUNG

Bekämpfung pflanzenparasitärer Nematoden 3.
Die finale Nematodendichte des Bodens als Mittel zum Abschätzen der Wirkung eines Begasungsmittels

Frühere Arbeiten über die Kurven für die Dosierungsreaktionen des Kartoffelälchens werden diskutiert. Da es schwierig ist, die Resultate von Versuchen mit Wurzeldiffusaten richtig zu erklären, wird eine neue Methode vorgeschlagen, die auf die finale Nematodendichte begründet ist. Erdfreie, lufttrockene Zysten wurden in geschlossenen Stahlkammern mit Methylbromid bei einer Reihe von Dosierungen begast, die als Produkte von Konzentration und Zeit in mg x Stunden je Liter gemessen wurden. Die Methoden, nach denen die Mortalität geschätzt wurde, werden beschrieben.

Es wird für gut gehalten, das Kriterium „neue Zysten je 200 g" dem Kriterium der fundamentalen Nematodeneinheit „Larven je g" vorzuziehen.

Die finale Nematodendichte ist wahrscheinlich nur für diejenigen Arten von *Heterodera* verwendbar, die eine vollständige, jährliche Generation haben, wie *H. göttingiana, H. avenae* und *H. rostochiensis,* aber möchte ausgedehnt werden zu Arten, die jährlich mehr als eine Generation haben.

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SOIL SAMPLE EXAMINATION AS A BASIS FOR ADVISORY WORK AGAINST STEM EELWORMS, *DITYLENCHUS DIPSACI* (KÜHN)

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Onion sets and onions for pickling are grown in the Netherlands on clay soils which are often infested with stem eelworms *Ditylenchus dipsaci* (Kühn). For these crops, attempts are made by soil sampling to select fields with very low stem eelworm populations. Preliminary surveys indicated that the levels of infestation which cause damage to onions for pickling are very low in practice. They can however be detected in field samples if the sampled area is not too large (at least one sample to each third of a hectare) and if a relatively large amount of soil from the sampled area is examined.

In the winter of 1958/59 the number of stem eelworms was determined in about 2,300 samples. A still higher number of samples will probably be examined in future and it is therefore important that examination should be rapid without loss of reliability.

METHODS

Field sampling and laboratory extraction

Each field to be examined is divided into areas of one third hectare and from each area sixty prods are taken to a depth of 20 cm to give approximately 1 kg of soil. The samples are processed whole to avoid the problems associated with mixing and dividing the heavy clay soil. To increase the efficiency of the laboratory examination, attention must be paid to the following points:

(1) The rapid isolation of the nematodes from the soil with as small a loss as possible.
(2) Obtaining as clear a suspension of nematodes as possible.
(3) The elimination of species other than *Ditylenchus dipsaci* from the suspension.

Oostenbrink's flotation funnel (OOSTENBRINK unpublished; GOFFART 1959) is used. Before putting the sample in this apparatus, a suspension in water must be made. This is done in an automatic stirrer (Fig. 1). In the mixing basin of this apparatus (capacity 5 l) one litre of water is added to 1 kg of soil. Homogeneous

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suspensions of light clay soil are obtained in 5 minutes and of heavy clay soil in 10 minutes. The stirrer in the bowl rotates at 100 revolutions per minute and the nematodes are not damaged by this treatment.

Normally, the contents of the flotation funnel is sieved through two 50 μ sieves, one placed on top of the other. When a kg of clay soil is put into the apparatus, sometimes a large mass of small soil particles is retained on these sieves.

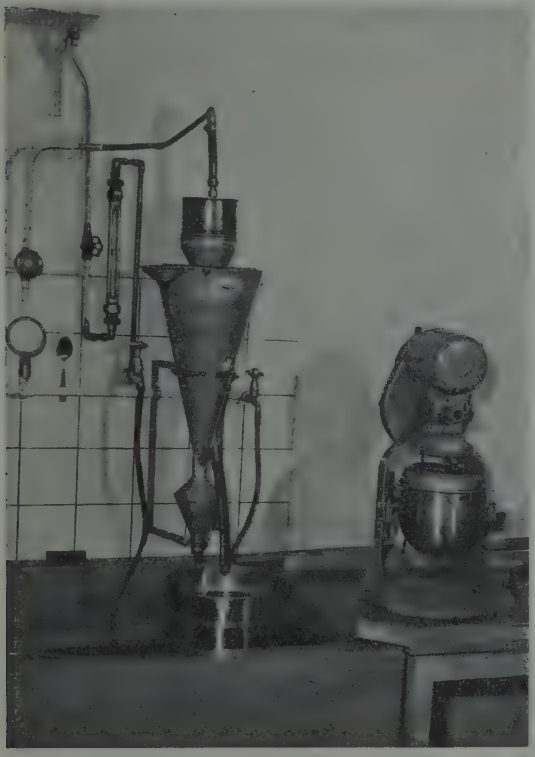

Fig. 1. Flotation funnel (left) and mechanical stirrer (right). Soil samples of 1 kg are suspended in water in the stirrer, after which the nematodes are extracted in the flotation funnel.

Most of it does not pass through the cotton wool filter used in the final separation (OOSTENBRINK, unpublished; GOFFART 1959), but the final nematode suspension may nevertheless be too cloudy. As a reduction of the water current in the funnel might result in lower extraction of nematodes, attempts were made to get rid of these fine particles by sieving through coarser sieves. In this way also some of the other species of nematodes smaller than stem eelworms were eliminated. With 75 μ and 105 μ sieves very clean suspensions were obtained. Table I gives the results of a comparison of sieving nematode suspensions obtained from

500 g of light clay soil through banks of two 50 μ, two 75 μ and two 105 μ sieves. The suspensions passing through these sieves were then passed through a bank of four 50 μ sieves, and in this way the number of nematodes lost by the first sieving was determined. The water passing through the cotton wool filter was poured on a second filter in order to determine the loss at this stage of the procedure. The loss in the apparatus was also investigated by processing the residues from the bottom and walls for a second time. This time the suspension obtained was sieved through a bank of four 50 μ sieves. The results in Table I are the mean sum of

TABLE I

Influence of the sieve mesh on the numbers of nematodes extracted from 500 g of a light clay soil

Mesh of the sieves	50 μ	75 μ	105 μ
Catch on 2 sieves	6810 (73 %)	5900 (58 %)	2935 (31 %)
Loss through sieves	1340 (14 %)	3100 (31 %)	5610 (58 %)
Loss in apparatus	880 (10 %)	830 (8 %)	915 (9 %)
Loss through filter	270 (3 %)	285 (3 %)	160 (2 %)
Total	9300	10115	9620

three experiments. Losses in the apparatus and through the filter appear to be fairly constant (11-13 %). However, the wider the meshes of the sieves used the larger the number of nematodes passing through.

In the same way 500 g samples containing stem eelworms were studied. As it was difficult to obtain samples with a high natural infestation, stem eelworms from diseased onions were added to soil for this purpose. Samples taken in winter do not contain stem eelworms smaller than 600 μ. Therefore, to obtain an impression of the losses when such samples are investigated, stem eelworms longer and shorter than 600 μ were counted separately. The results are recorded in Tables II and III. The loss through the sieves was determined here by catching the fil-

TABLE II

Influence of the sieve mesh on the number of stem eelworms extracted

Mesh of the sieves	50 μ	75 μ	105 μ
Catch on 2 sieves	4465 (95 %)	5080 (92 %)	5500 (89 %)
Loss through sieves	27 (0.6 %)	273 (5 %)	515 (8 %)
Loss in apparatus	170 (4 %)	185 (3 %)	187 (3 %)
Loss through filter	12 (0.3 %)	3 (0.1 %)	4 (0.1 %)
Total	4674	5541	6206

TABLE III

Influence of the sieve mesh on the number of stem eelworm larvae extracted, smaller than 600 μ

Mesh of the sieves	50 μ	75 μ	105 μ
Catch on 2 sieves	1485 (88 %)	1120 (70 %)	960 (54 %)
Loss through sieves	80 (5 %)	383 (24 %)	690 (39 %)
Loss in apparatus	77 (4 %)	70 (4 %)	104 (6 %)
Loss through filter	47 (3 %)	39 (2 %)	24 (1 %)
Total	1689	1612	1778

trate on two 50 μ sieves and following the same procedure as in the first set of experiments. Only a few small stem eelworms passed through the cotton wool filter. Also the loss of stem eelworms in the apparatus was smaller than that of other nematodes.

The losses of stem eelworms longer than 600 μ through a bank of two sieves of 50 μ, 75 μ and 105 μ were 0.6 %, 5 % and 8 % respectively (total loss of nematodes 14 %, 31 % and 58 % respectively). More small nematodes (< 600 μ) passed through the sieves and the filter and were lost in the apparatus than larger nematodes (> 600).

The loss of stem eelworms when sieving through 75 μ sieves and even when using 105 μ sieves is small, and the numbers of many other species of nematodes are reduced. Therefore the use of 75 μ or 105 μ sieves is preferred to 50 μ sieves. For the collection of nematodes other than stem eelworms (e.g. *Pratylenchus*) a bank of four 50 μ sieves is necessary.

The effect of drying nematodes in the cotton wool filters

Stem eelworms withstand desiccation though this kills many other species. Therefore drying the nematodes recovered from the soil was tried as a means of eliminating other species and simplifying the counting. Drying the whole soil sample is impracticable but the cotton wool filter with the nematodes can be dried immediately after filtering the nematode suspension. To obtain the right rate of drying a chest of drawers was constructed in which twenty filters could be placed (Fig. 2). Air was blown through this chest by a fan, the amount being regulated from 1 to 30 m^3 an hour per sample. At the back of the chest the air was distributed equally over the drawers each of which held four filters. The air passes through holes just above the cotton wool filters and escapes through slits in the sides of the chest.

As soon as the filters were dry and drought sensitive nematodes killed, enough water was added to the trays to fill the space below the filters and moisten them. The stem eelworms then became active again, and passed through the filter into the water in the tray. Ninety nine percent of the nematodes other than stem eelworms were killed by this means.

A high recovery of stem eelworms can be obtained only when the filter is dried gradually (in not less than 10 hours) at 20° C and a relative humidity of 40-50 %. This is obtained by blowing about 5 m^3 of air an hour per sample through the chest if each filter contains about 90 ml of water.

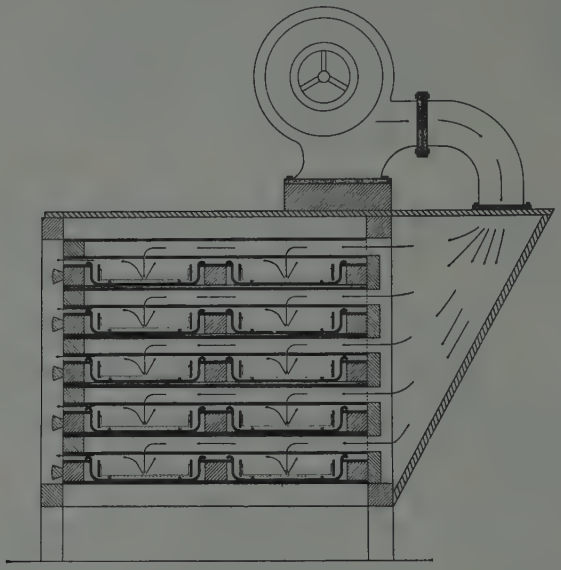

Fig. 2. Cross-section of the apparatus for drying the cotton-wool filters. Cf. text.

TABLE IV

The effect of drying the cotton-wool filter on the recovery of stem eelworms from soil samples of 1 kg

Nr.	Number of samples	Temp. (° C)	Rel. humid. (%)	Number of stem eelworms before drying	Number of stem eelworms after drying
1	5	± 20	45-60	66	57 (86 %)
2	19	± 20	30-55	280	253 (90 %)
3	9	20-30	± 40	73	52 (71 %)
4	5	16-23	± 40	146	163 (> 100 %)
5	7	± 20	± 40	95	88 (93 %)
6	9	± 20	± 40	537	491 (91 %)
7	8	± 20	± 40	3333	3234 (97 %)
8	10	± 20	± 40	4758	3712 (80 %)

Table IV compares the results of normal treatment with drying in sixty three soil samples of 1 kg. Drying appears to reduce the number of stem eelworms recovered only slightly. The low recovery after drying of Nr. 4 (Table IV) is

probably due to drying at too high a temperature. More experiments are necessary to determine the range of temperatures and relative humidities at which good results can be expected.

ZUSAMMENFASSUNG

Untersuchung von Bodenproben als Grundlage für den Beratungsdienst gegen Stengelälchen Ditylenchus dipsaci (*Kühn*)

In Zwiebelkulturen ist für Beratungszwecke eine Bodenuntersuchung auf Stockälchen wichtig. Eine Bodenmischprobe wird von 1/3 ha mit einem engen Bohrer aus 20 cm Tiefe gezogen. Sie besteht aus etwa 60 Einstichen und soll etwa 1 kg Boden betragen. Die Proben werden mit dem von Oostenbrink entwickelten Gerät ausgewaschen. Zur Prüfung großer Bodenmengen (1 kg) wurden einige Veränderungen vorgenommen.

Versuche wurden mit zwei Sieben von 50, 75 und 105 μ Maschenweite durchgeführt. Wenn die Nematoden nach dem Ausschlämmen durch einen Satz von zwei Sieben erhalten wurden, lagen die Verluste bei 0.6, 5 und 8 %. Die Verluste bei anderen Nematodenarten betrugen 14, 31 und 58 %. Mit großen Proben von schwerem Lehmboden gaben die mit den 75 und 105 μ-Sieben gesammelten Nematoden endgültig klarere Suspensionen als mit den 50 μ-Siebe und sind daher zu bevorzugen. Ein zusätzlicher Vorteil ist, daß ein Teil der anderen Nematoden entfernt wird.

Es scheint möglich zu sein, Stengelälchen automatisch von anderen trockenempfindlichen Arten zu trennen durch Austrocknenlassen der Baumwollfilter. Mehr als 90 % der Stengelälchen, die während des Herbstes und Winters im Boden vorhanden sind, scheinen diese Behandlung zu überstehen, während mehr als 99 % der anderen Nematoden abgetötet werden, sodaß dieses Verfahren die Möglichkeit einer Wirkungssteigerung bei Stengelälchen bietet.

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UNTERSUCHUNGEN ÜBER DAS AUFTRETEN FREILEBENDER NEMATODEN IN FRUCHTFOLGEN (VORLÄUFIGE ERGEBNISSE)

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Die Zahl der in Fruchtfolgen auf die Zusammensetzung der Nematodenfauna einwirkenden Faktoren ist so groß und variabel, daß die Beurteilung eines Nematodenbestandes sehr erschwert wird. Die Faktorenkomplexe Bodenklima, Mikroorganismen und organische Substanzen sind in jeder Bodenart die wichtigsten, weil sie die Entwicklung der Nematoden steuern (Entwicklungsgeschwindigkeit, Ernährungsgrundlage). Sobald ein Pflanzenbestand das Feld räumt, entfällt sein regulierender Einfluß auf die Geschehnisse im Boden. Klimatische Faktoren schwanken stärker, die absterbenden Pflanzenwurzeln erhöhen kurzfristig die Menge der organischen Substanzen. Es konnte mehrfach beobachtet werden, daß nach Umbruch von Rotklee, Weizen und Roggen sich die Besatzzahlen von *Helicotylenchus sp.* und *Pratylenchus sp.*, aber auch von *Acrobeloides sp.* und anderen Saprophagen stark veränderten. Durch die Bodenbearbeitung nach der Ernte werden neue Entwicklungsgrundlagen für alle Bodenlebewesen geschaffen, die aber auf Grund der tiefen Temperaturen während des Winters eingeengt werden. Es gibt Nematodenarten (z. B. *Panagrolaimus rigidus*), die sich in dieser Zeit unabhängig von der Art des Pflanzenbestandes stark vermehren können.

Sobald die Vegetation im Frühjahr einsetzt, bildet der neue Pflanzenbestand sein eigenes Bestandesklima aus. Mit zunehmendem Wachstum der Pflanzen und gleichzeitig einsetzender Aktivierung der Bodenfauna und -flora ändert sich auch die Zusammensetzung der Nematodenfauna. Es ist mit Sicherheit anzunehmen, daß Bodenklima, Mikroorganismen und organische Substanzen (bei obligaten Parasiten auch die Pflanzen) diejenigen Faktoren sind, die zu diesem Zeitpunkt die qualitative und quantitative Umformung der Nematodenfauna verursachen.

Die Art des Einflußes der Temperatur auf die Nematodenvermehrung (direkt über die Entwicklungsdauer oder indirekt über die Mikroorganismen) bleibt noch zu klären. Experimentelle Untersuchungen ergaben, daß Mikroorganismenbesatz und organische Substanzen komplex die freilebenden Nematoden quantitativ und qualitativ außerordentlich intensiv beeinflußen.

Bedeutsam für freilebende Nematoden in Fruchtfolgen ist die Düngung mit Stalldünger. Nach Franz (1953) vermindert frischer Stalldünger die Nematodenzahlen, die nach einiger Zeit wieder steigen. Je besser die Verrottung, desto kürzer die Depression. Auf mitteldeutschem Löss-Lehm konnte nachgewiesen werden, daß bei gut verrottetem Dünger diese Depression noch im Jahre nach der Düngung andauern kann. Auf solchen Flächen herrschten die Saprophagen, besonders *Acrobeloides bütschlii,* vor. Weiterhin muß die Vermutung ausgesprochen werden, daß auf genanntem Boden Kartoffeln mit Stalldünger einen positiven Einfluß auf die Vermehrung von *Pratylenchus sp.* im folgenden Jahre ausüben (vergleiche Oostenbrink u. a. 1956).

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UNTERSUCHUNGEN ÜBER DAS AUFTRETEN PFLANZENPARASITÄRER NEMATODEN IN WEINBERGSBÖDEN

VON

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Der Weinbau ist in Deutschland fast 2000 Jahre alt. Die von der Rebe für qualitativ und quantitativ gute Erträge geforderten Wachstumsbedingungen sind aber nur an wenigen Stellen des Landes erfüllt. Die Hauptanbaugebiete erstrecken sich längs des Rheins vom Bodensee bis Bonn und umfassen das Rheintal und die Täler von Neckar, Main, Nahe, Mosel, Lahn und Ahr. An einigen dieser Stellen wird in nahezu ununterbrochener Folge seit Jahrhunderten Weinbau getrieben. Ein geregelter Fruchtwechsel wird nicht durchgeführt. Die Stöcke stehen in der Regel 30-50 Jahre, und meist wird eine kurze Brache sowie ein tiefes Rigolen (60-100 cm tief) nach dem Aushauen der alten Stöcke als ausreichend für die Erholung des Bodens angesehen. Die Nematodenfauna dieser extremen Monokulturen ist in Deutschland bisher noch nicht planmäßig untersucht worden. Auch aus anderen europäischen Weinbaugegenden liegen nur sehr vereinzelte Beobachtungen vor, in denen die freilebenden Formen z.T. nicht einmal berücksichtigt sind (Menzel 1940, 1941; Goodey, 1940; Goffart, 1951; Ritter, 1953; Boubals, 1954; Weischer, 1957; Ciccarone, 1959; Hopp, 1959).

Es erschien daher angebracht, eine umfassende Untersuchung über das Auftreten pflanzenparasitärer Nematoden einzuleiten, zumal in den letzten Jahren in anderen Ländern erhebliche Schäden durch freilebende Nematoden an Wein nachgewiesen werden konnten (Raski, 1954, 1955; Seinhorst & Sauer, 1956). Über die ersten Ergebnisse der Erhebungen soll hier berichtet werden.

Da es unmöglich war, sofort das gesamte deutsche Weinbaugebiet zu erfassen, wurde zunächst das Moseltal als Schwerpunkt gewählt mit einigen Untersuchungsstellen in anderen Flußtälern (Abb. 1). Die untersuchten Weingärten liegen alle an den mehr oder weniger steilen Hängen der Flußtäler, wo sie vor kalten Winden geschützt sind. Die Böden sind von unterschiedlicher geologischer Herkunft und enthalten meist einen hohen Prozentsatz von Steinen. Schieferverwitterung mit durchschnittlich 30-40 % Feinerde findet sich an der unteren und mittleren Mosel, an der Ahr und an der Lahn. In Vallendar (Rhein) besteht der Boden aus Grauwackeverwitterung mit etwa 30 % Feinerde und an der oberen Mosel (Langsur und Nittel) aus schwerer Muschelkalkverwitterung mit wenigen Steinen (Zillig &

Abb. 1. Die wichtigsten deutschen Weinbaugebiete (in den Flußtälern). An den durch + gekennzeichneten Orten wurden Bodenuntersuchungen durchgeführt.

HERSCHLER, 1931). Der pH-Wert liegt für die meisten Böden in alten Rebanlagen im neutralen bis schwach saurem Bereich (pH 6,6), auf dem Kalkboden dagegen bei pH 7,5 und höher.

Neben dem Boden ist das Klima sehr wichtig für die Kultur von Reben. Da die Weinrebe sehr wärmebedürftig ist und für gute Erträge eine starke Sonneneinstrahlung benötigt, liegen die Weinberge möglichst nach Süden. In diesen Biotopen herrschen gegenüber dem Flachland sehr veränderte Bedingungen, die auch die Nematodenfauna in charakteristischer Weise beeinflußt haben. Zum Studium der Nematodenfauna wurden an den in Abb. 1 gekennzeichneten Orten zu verschiedenen Jahreszeiten Bodenproben entnommen und mit dem Seinhorst-Elutriator auf freilebende Nematoden untersucht. Außerdem wurden die Wurzeln auf das Vorhandensein von Wurzelgallenälchen (*Meloidogyne* spp.) geprüft. Diese Nematoden spielen im Weinbau südeuropäischer Länder eine wichtige Rolle als Schädlinge. In unserem Untersuchungsgebiet wurden sie bisher noch nicht gefunden. Wahrscheinlich sind die verhältnismäßig schweren Böden für diese Nematoden ungünstig. In Tabelle I sind zunächst die bisher festgestellten Nematoden, soweit sie als Pflanzenparasiten in Betracht gezogen werden können, und ihre Verbreitung wiedergegeben. Dabei, sowie auch in den nächsten Abschnitten, werden vorerst nur Unterschiede zwischen den einzelnen Gattungen besprochen. Die Eigenschaften der verschiedenen Arten werden weiter unten behandelt. Die Zusammenstellung in Tabelle I enthält 9 Gattungen, die alle zu den wandernden Wurzelnematoden gerechnet werden können. Auf Grund der Verbreitung heben sich 3 Gattungen von den übrigen ab: *Paratylenchus, Criconemoides* und *Helicotylenchus*. Vertreter dieser Genera wurden fast im ganzen Untersuchungsgebiet gefunden und scheinen im Weinbau weit verbreitet zu sein. Von den übrigen Gattungen kamen *Pratylenchoides* und *Xiphinema* an mehreren Stellen vor, während der Rest nur in einzelnen Gebieten auftrat. Trotzdem können diese Nematoden nicht sämtlich als Zufallsfunde bezeichnet werden, denn ihre Verbreitung richtet sich z.T. offenbar streng nach der Bodenart, wie sich im Laufe der Untersuchungen gezeigt hat.

Über die Populationdichte der einzelnen Formen gibt Tabelle II Auskunft. Da die Nematoden an den verschiedenen Orten und in den verschiedenen Bodentiefen unterschiedlich häufig waren, wurden die Werte von insgesamt 17 Untersuchungsstellen zu Durchschnittswerten zusammengefasst. Die Tabelle ergibt daher nur ein stark vereinfachtes Bild, das aber einen Eindruck von den Größenordnungen vermittelt. Die bei weitem größten Populationen hat *Paratylenchus*. Sie sind in der Regel fast zehnmal so stark wie die der anderen Nematoden. Diese Erscheinung ist aber nicht auf die Weinberge beschränkt. Auch in anderen Kulturen findet man oft, daß *Paratylenchus* zahlenmäßig andere pflanzenparasitäre Älchen um ein Mehrfaches übertrifft. *Criconemoides* ist im Weinbaugebiet ebenfalls weit verbreitet. Er findet sich aber selten in größeren Mengen. Nach den Beobachtungen bestehen bei diesen Nematoden große Unterschiede in der Häufigkeit an den verschiedenen Fundorten. An einzelnen örtlich eng begrenzten Stellen findet

TABELLE I

Verbreitung pflanzenparasitärer Nematoden im Weinbaugebiet von Mosel, Rhein, Lahn und Ahr

\+ = gefunden, 0 = nicht gefunden

NEMATODEN	UNTERSUCHUNGSORTE									
		Mosel				Rhein	Lahn		Ahr	
	Pommern	Zell	Bernkastel	Langsur	Nittel	Vallendar	Ems	Obernhof	Ahrweiler	Dernau
Paratylenchus	+	+	+	+	+	+	+	+	+	+
Criconemoides	+	+	+	+	0	+	+	+	+	+
Helicotylenchus	+	+	+	+	+	+	+	+	0	+
Pratylenchoides	+	0	+	+	+	+	0	0	0	0
Xiphinema	+	0	0	0	0	0	0	+	0	+
Gottholdsteineria	0	0	0	+	0	0	0	0	0	0
Pratylenchus	0	0	0	+	0	0	0	0	0	0
Macrotrophurus	0	0	0	0	+	0	0	0	0	0
Hemicycliophora	0	0	0	0	0	+	0	0	0	0

TABELLE II

Populationsstärke pflanzenparasitärer Nematoden in Weinbergen.
Die Werte beziehen sich auf 250 ccm Erde

NEMATODEN	POPULATIONSSTÄRKE		NEMATODEN	POPULATIONSSTÄRKE
	normal	maximal		maximal
Paratylenchus	3,720	12,410	*Gottholdsteineria*	530
Criconemoides	460	3,490	*Pratylenchus*	490
Helicotylenchus	380	1,190	*Macrotrophurus*	40
Pratylenchoides	400	1,180	*Hemicycliophora*	90
Xiphinema	25	35		

man große Populationen (mehrere tausend Tiere in 250 ccm Boden). Im allgemeinen sind sie aber wesentlich geringer. Der Unterschied zwischen dem Normalwert und dem Maximalwert in Tabelle II macht das deutlich. Die Tiere scheinen im Boden nur wenig zu wandern. Im Gegensatz dazu sind bei *Helicotylenchus* die Unterschiede in der Häufigkeit an den Untersuchungsorten wesentlich geringer. Die Tiere finden sich nirgends in besonders hohen Anzahlen, aber ebenso selten in sehr kleinen. Sie sind im ganzen Gebiet ziemlich gleichmäßig verteilt. Ähnliches gilt auch für die übrigen Formen, soweit sie überhaupt an mehreren Stellen gefunden wurden. Ob die auffallend geringen Populationen von *Xiphinema* und *Macrotrophurus* den tatsächlichen Verhältnissen entsprechen, bedarf noch der Nachprüfung. Es ist nicht ausgeschlossen, daß bei der bisher angewandten Technik der Probenahme und des Auswaschens diese großen Nematoden nicht restlos erfaßt worden sind.

Neben der geographischen Verbreitung wurde auch die vertikale Verteilung der Nematoden im Boden untersucht. An 8 verschiedenen Stellen wurden Bodenproben aus 5-10, 25-30, 45-50 und 65-70 cm Tiefe entnommen und auf Nematoden untersucht. Im allgemeinen ist die größte Älchendichte in den oberen Schichten bis etwa 20 cm Tiefe zu finden (OVERGAARD NIELSEN, 1949). Das trifft auch für die Weinberge zu, sofern man die Nematodenfauna insgesamt betrachtet. Wie Tabelle 3 zeigt, sind die oberen Schichten wesentlich dichter besiedelt als die tieferliegenden. Der größte Sprung liegt dabei meistens zwischen der ersten und der zweiten Stufe. Eine Ausnahme machten die Proben aus einem Weinberg in Vallendar (Rhein), bei denen zwei deutliche Maxima der Häufigkeit vorhanden waren. Bei näherer Untersuchung zeigte sich, daß sehr wahrscheinlich die etwas abweichende Bewurzelung der Reben an diesem Hang diese Verteilung bewirkt hat. Das für die Gesamtnematodenfauna Gesagte gilt auch für die pflanzenparasitären Gattungen mit Ausnahme von *Paratylenchus*. Die Tabelle IV gibt einige konkrete Beispiele für die vertikale Verteilung dieser Nematoden wieder. Es ist sofort zu sehen, daß *Paratylenchus* sich anders verhält als die übrigen angeführten Gattungen. Diese sind, wie die meisten freilebenden Formen, in den oberen Schichten am häufigsten und nehmen nach unten hin schnell ab. Oft sind in der untersten der untersuchten Schichten keine oder nur vereinzelte Tiere dieser Gattungen vorhanden. Bei *Paratylenchus* dagegen ist es umgekehrt. Hier leben in den oberen Schichten nur wenige Tiere, während die Hauptmasse in größerer Tiefe ist. Bei einem Vergleich der Verteilung zu verschiedenen Jahreszeiten zeigte sich, daß die geschilderte vertikale Schichtung im Herbst (September) am deutlichst ist, während im Frühjahr (März) die Grenzen nicht so klar zu sehen sind. Dann findet man auch in den oberen Schichten mehr Paratylenchen. Im Juni macht sich dann wieder die Tendenz zur Schichtung bemerkbar. Diese Beobachtungen lassen darauf schließen, daß bei *Paratylenchus* die Feuchtigkeit maßgebend für die Verteilung ist, wobei wahrscheinlich die geringeren Schwankungen in größeren Tiefen ebenso wichtig sind wie der Wassergehalt schlechthin. Auch in anderen Kulturen und auf anderen Böden haben wir *Paratylenchus* bevorzugt an

TABELLE III

Vertikale Verteilung der Nematoden in Weinbergsböden. Die Zahlen sind Durchschnittswerte aus verschiedenen Proben und geben die Gesamtheit der Nematoden in jeweils 250 ccm Erde wieder.

BODENTIEFE	UNTERSUCHUNGSORTE							
		Mosel			Rhein	Lahn	Ahr	
in cm	Pommern	Bernkastel	Langsur	Nittel	Vallendar	Obernhof	Dernau	Ahrweiler
5-10	3 010	11 350	6 036	6 890	10 930	1 040	4 200	10 610
25-30	1 100	4 800	2 310	2 210	760	590	2 510	6 100
45-50	580	3 210	1 810	2 130	4 680	2 320	2 320	2 720
65-70	450	1 430	350	1 040	920	310	670	1 400

TABELLE IV

Verteilung von Paratylenchus, Criconemoides, Helicotylenchus *und* Pratylenchoides *in verschiedenen Bodentiefen an vier Fundorten. Pom. = Pommern (Mosel), Lan. = Langsur (Mosel), Val. = Vallendar (Rhein), Der.= Dernau (Ahr)*

Bodentiefe	*Paratylenchus*				*Criconemoides*		*Helicotylenchus*		*Pratylenchoides*	
in cm	Pom.	Lan.	Val.	Der.	Pom.	Lan.	Lan.	Der.	Lan.	Val.
5-10	0	368	24	7	430	360	1 948	1 190	502	1 203
25-30	10	720	183	240	77	30	760	535	490	190
45-50	116	920	1 580	660	12	25	220	170	410	22
65-70	973	70	910	1 530	0	6	85	0	60	0

Orten mit gleichmäßiger Feuchte angetroffen, während er selten in Böden mit stark schwankendem Wassergehalt auftrat. Demgegenüber scheint die Durchlüftung des Bodens, die für die meisten Nematoden sehr wichtig ist, für *Paratylenchus* an Bedeutung zurückzutreten. Ähnliches gilt in Weinbergsböden offenbar auch für *Xiphinema*. Diese Nematoden wurden nur in Tiefen unter 45 cm gefunden. Die Verteilung der anderen hier untersuchten Pflanzenparasiten wird wohl in erster Linie durch die Sauerstoffversorgung im Boden bestimmt. Trockenheit und Wärme in den oberen Schichten scheinen keinen allzu großen Einfluß zu haben. Ob und wieweit morphologische Eigenschaften, wie z.B. Körpergröße, Dicke der Kutikula usw. oder auch Stoffwechseleigentümlichkeiten bei den unterschiedlichen Reaktionen der genannten Nematoden eine Rolle spielen, ist bisher noch nicht untersucht worden. Überhaupt sind die ökologischen Ansprüche der verschiedenen Nematoden im Einzelnen noch weitgehend unbekannt.

Bei den vorstehend geschilderten Untersuchungen ist nur allgemein von den Gattungen die Rede gewesen ohne Berücksichtigung der Arten. Die genaue taxonomische Bearbeitung der in den Weinbergen gefundenen Nematoden wird noch einige Zeit in Anspruch nehmen, zumal einige neue Arten zu untersuchen und zu beschreiben sind. Das soll in einer anderen Veröffentlichung geschehen. Hier sei nur soweit auf diese Frage eingegangen, wie es zur Charakterisierung der Verhältnisse notwendig und wichtig erscheint.

1. *Paratylenchus*

Im untersuchten Gebiet kommen vier Formen vor, die sich morphologisch gut unterscheiden lassen. Da eine genaue Determination noch nicht möglich ist und die neuen Arten erst beschrieben werden müssen, wurde zunächst eine einfache Einteilung nach der Mundstachellänge vorgenommen. Die Verbreitung der einzelnen Formen wird hauptsächlich durch die Bodenbeschaffenheit bestimmt. Eine Art, die durch einen 90 μ langen Mundstachel gekennzeichnet ist, findet sich nur in dem schweren, dichten Lehmboden am Oberlauf der Mosel. Zwei weitere Arten, eine mit einem gebogenen Mundstachel von 79 μ und die andere mit einem kurzen Mundstachel von 17 μ Länge, kommen ebenfalls in schweren Lehmböden vor, aber nur dort, wo sie durch einen höheren Gehalt an Steinen und groben Teilchen nicht so dicht sind. Die größte Verbreitung hat eine Form mit einem 29 μ langen Mundstachel. Sie wurde in allen Schiefer- und Grauwackeböden der untersuchten Flußtäler gefunden. Wahrscheinlich ist es aber keine einzelne Art, sondern eine Gruppe einander sehr ähnlicher Arten.

2. *Criconemoides*

Soweit bisher festgestellt werden konnte, kommt im untersuchten Gebiet nur eine Art vor, *Criconemoides xenoplax* Raski, 1952. Männchen sind selten, konnten aber vor allem im Frühjahr wiederholt gefunden werden. *C. xenoplax* war an

allen Stellen vertreten, mit Ausnahme des schweren dichten Lehms in Nittel. Nach den bisherigen Beobachtungen im Freiland und im Laboratorium ist das Fehlen an dieser Stelle auf die geringere Durchlüftung des bindigen Bodens zurückzuführen.

3. *Pratylenchoides*

Bei den Untersuchungen wurden an mehreren Stellen Vertreter der Gattung *Pratylenchoides* gefunden. Die Artzugehörigkeit ist noch nicht bestimmt. Von der einzigen bisher beschriebenen Art *Pratylenchoides crenicauda* Winslow, 1958 unterscheiden sie sich unter anderem durch die Form der Speicheldrüse und durch das Vorhandensein von zwei Spermatheken bei den Weibchen. Die Tiere wurden sowohl in dem schweren Kalkboden der oberen Mosel als auch in den leichteren, steinigeren Böden des übrigen Moseltales und des Rheins gefunden.

4. *Pratylenchus*

Vertreter der Gattung *Pratylenchus* konnten bisher nur an einer Stelle (Langsur) auf Kalkboden in der Nähe der Grenze zur Schieferverwitterung gefunden werden. Die Art ist nahe verwandt oder identisch mit *P. thornei* Sher & Allen, 1953. Irgendwelche Ursachen für dieses eng begrenzte Vorkommen konnten noch nicht ermittelt werden. Durch Ausdehnung des Untersuchungsgebietes und durch eine intensivere Nachforschung in der Umgebung des Fundortes sollen weitere Unterlagen beschafft werden.

5. *Xiphinema*

Die gefundenen Tiere gehören zu einer Art, die nahe verwandt ist mit *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939 und *X. index* Thorne & Allen, 1950. Die morphologischen Merkmale stimmen mit keiner der beiden Arten genau überein, doch ist die Ähnlichkeit mit *X. diversicaudatum* am größten. Im untersuchten Weinbaugebiet war die Verbreitung der Tiere auf Schieferböden beschränkt. In dem schweren, wenig durchlüfteten Lehm an der Obermosel wurden sie nicht gefunden. Das Vorkommen an weit auseinanderliegenden Stellen läßt auf ein größeres Verbreitungsgebiet schließen. Im allgemeinen scheint die Gattung in Europa aber nicht häufig zu sein. In Deutschland wurde sie bisher außer an Reben von uns nur einmal an Erdbeeren festgestellt.

6. *Macrotrophurus*

Bei den gefundenen Tieren handelt es sich wahrscheinlich nicht um die bisher einzige Art *Macrotrophurus arbusticola* Loof, 1958. Die jüngeren Larven haben nämlich kein einfach abgerundetes Hinterende wie die Weibchen. Es ist vielmehr unregelmäßig ausgebildet und zeigt nach einer Verengung eine oft keulenartige Anschwellung. Nach Loof (mündl. Mitteilung) wurden diese Älchen vor einiger Zeit auch in Holland gefunden. In den Weinbergen konnten die Tiere nur auf

schwerem Lehm festgestellt werden. Die Populationen waren verhältnismäßig klein.

7. *Helicotylenchus* und *Gottholdsteineria*

Die Klärung der artenmäßigen Zusammensetzung dieser Nematoden wurde durch das Vorkommen von Mischpopulationen erschwert und konnte bisher noch nicht befriedigend durchgeführt werden.

8. *Hemicycliophora*

Die Artbestimmung war mangels einer ausreichenden Menge erwachsener Tiere noch nicht möglich. Es scheint sich hier nur um ein zufälliges Auftreten ohne phytopathologische Bedeutung zu handeln, da die Tiere nur an einem einzigen Rebstock festgestellt wurden.

Zum Schluß sei noch auf die Bedeutung der gefundenen Nematoden als Weinbauschädlinge eingegangen, soweit sie nach den vorliegenden Beobachtungen beurteilt werden kann. Im Untersuchungsgebiet wurden an etlichen Stellen nesterartige Wachtumsdepressionen festgestellt, die teils mit besonderen Symptomen wie Blattdeformationen, Panaschüren, Triebstauchungen usw. verbunden sind, teils aber auch nur ein allgemein schlechtes Wachstum aufwiesen. Diese Fehlstellen breiten sich im Laufe mehrerer Jahre langsam aus. Fast immer ist das Wurzelsystem der „kranken" Stöcke schlecht entwickelt und teilweise im Absterben. In vielen Fällen konnte hier ein gegenüber gesunden Reben verstärkter Besatz an pflanzenparasitären Nematoden nachgewiesen werden. Bei älteren (größeren) Herden waren an den noch gesund erscheinenden Reben der Randzone mehr Nematoden als in der Mitte. Diese bei Älchenbefall nicht seltene Erscheinung hängt hauptsächlich mit dem stark reduzierten Wurzelsystem der Pflanzen in Herdmitte zusammen. Die geschilderten Wachstumsdepressionen treten in schlecht gepflegten und stark ausgenutzten Weinbergen häufiger auf als in gut versorgten Anlagen. Ähnliche Beobachtungen wurden von RASKI (1955) in Kalifornien gemacht, wo die Höhe der Nematodenschäden an Reben wesentlich von der Kulturart und Pflege abhing. Durch mikroskopische Untersuchung der Wurzeln und durch Infektionsversuche im Gewächshaus konnte bisher für *Paratylenchus* und *Criconemoides* nachgewiesen werden, daß sie die Rebwurzeln befallen und sich dort vermehren. Genaue Angaben über den Umfang der durch diesen Befall verursachten Schäden könnten noch nicht gemacht werden. Die Nematoden vermehrten sich bei uns merklich langsamer als bei den in Kalifornien durchgeführten Untersuchungen von RASKI & RADEWALD (1958), sodaß längere Beobachtungszeiten erforderlich sind. An den Wurzeln der infizierten Pflanzen wurden Nekrosen beobachtet, die offenbar aus den Saugstellen der Nematoden hervorgegangen waren. Neben diesem direkten Befall kommt den Nematoden neuerdings im Weinbau besondere Bedeutung als mögliche Vektoren bodenübertragbarer Viren zu (HEWITT, RASKI & GOHEEN, 1958). Diese vorerst nur für

Kalifornien bewiesenen Verhältnisse könnten auch für Deutschland zutreffen, nachdem die vorliegenden Untersuchungen das Vorkommen entsprechender Nematoden ergeben haben und auch hier bodenübertragbare Krankheiten viröser Natur bekannt sind (Hopp, 1959). Versuche zum Nachweis der Übertragung dieser Erreger durch Nematoden wurden eingeleitet.

Dem Leiter des Instituts für Rebenkrankheiten der Biologischen Bundesanstalt in Bernkastel-Kues (Mosel), Herrn Ob. Reg. Rat Dr. L. Niemeyer sei an dieser Stelle dafür gedankt, daß er die Arbeiten durch wichtige Hinweise und Hilfe bei der Materialbeschaffung ermöglicht und unterstützt hat.

SUMMARY

Observations on the occurrence of plant parasitic nematodes in the soil of vineyards

Vine growing in Germany in an example of extreme monoculture. The areas most suited by soil and climate are the slopes of valleys and here grape-vines have often been grown continuously for centuries. Soil samples from vineyards contained the following genera of plant parasitic or suspected parasitic nematodes: *Paratylenchus, Criconemoides, Helicotylenchus, Pratylenchoides, Xiphinema, Gottholdsteineria, Pratylenchus, Macrotrophurus* and *Hemicycliophora.* The first three genera occurred in all the places examined, five in the Mosel valley, two each in the Lahn and Ahr valleys and one in the Rhein valley, ten sites in all. *Pratylenchoides* was found in four out of ten sites and *Xiphinema* in three. Representatives of the last four genera were found in one site only. The vertical distribution of *Paratylenchus* in the soil was the opposite to that for the other genera. Whereas the latter were most common in the upper layers (5-10 cm.), *Paratylenchus* reached its maximum density in the layer below 50 cms. Study of seasonal fluctuations showed that for *Paratylenchus* the moisture content of the soil was the limiting factor; whereas the distribution of other nematodes such as *Criconemoides* depended more on oxygen supply. All forms encountered could not be named. There appeared to be one new species of *Pratylenchoides* and at least two new species of *Paratylenchus* with very long stylets (79 μ and 90 μ respectively). Root examination and greenhouse tests showed that *Paratylenchus* and *Criconemoides* live and multiply on vine roots as ectoparasites. The finding of *Xiphinema* in German vineyards raises the problem of virus transmission as in California.

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MEHRJÄHRIGE UNTERSUCHUNGEN ZUM WIRTSPFLANZENKREIS EINER HERKUNFT DES STOCK- UND STENGELÄLCHENS (*DITYLENCHUS DIPSACI*)

VON

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Im Gebiet der D.D.R. kommt das Stockälchen in mehreren Formen mit unterschiedlichen Wirtspflanzenkreisen vor. Sein Schadauftreten ist lokal begrenzt, obschon einzelne Nematoden dieser Art in sehr verschiedenen Bodenproben gefunden wurden. Abb. 1 zeigt Verteilung und Häufigkeit von Stockälchenschäden in den Jahren 1949-1958.

Die bevorzugt Roggen befallende Form verursacht regelmäßig nur in den Kreisen Kamenz und Bitterfeld Ertragseinbußen, eine Zwiebeln beeinträchtigende Form trat nach NOLTE (1956) in Bördegebiet stärker in Erscheinung. Der gelegentliche Befall von Kleearten ist wirtschaftlich ohne Bedeutung und bleibt auf das Küstengebiet sowie das Mittelgebirgsvorland begrenzt.

In den Jahren 1956-58 wurden durch die Biologische Zentralanstalt Berlin auf einem stark mit Stockälchen verseuchten Feld einer Landwirtschaftlichen Produktionsgenossenschaft im Kreise Kamenz ausgedehnte Erhebungen zum Wirtspflanzenkreis der dort auftretenden, bevorzugt Roggen schädigenden Herkunft von *Ditylenchus dipsaci* (Kühn) durchgeführt. 1955 lief bereits ein ähnlicher, kleinerer Versuch in einer Nachbargemeinde.

Auf etwa 6 cm großen Parzellen wurden 45 bzw. 60 Kulturpflanzenarten und -sorten angebaut. Ferner wurden etwa 20-25 Unkräuter von den Parzellen selbst oder aus unmittelbarer Nachbarschaft der Versuchsfläche in die Untersuchungen mit einbezogen.

Bewertet wurden außer sichtbaren Schädigungen die in gleichen Gewichtsmengen grüner Pflanzen enthaltenen Stockälchen. Je Parzelle wurden mindestens 4×5 g, meist aber 6-8 $\times$ 5 g Pflanzensubstanz nach der etwas abgewandelten Baermann'schen Trichter-Methode zu verschiedenen Zeiten untersucht, die gefundenen Älchen anzahlmäßig in Bewertungsgruppen eingeordnet sowie die in der Suspension enthaltenen Entwicklungsstadien bestimmt.

Die Befallsstärken der einzelnen Wirtspflanzen — als Kriterium diente das Vorhandensein reifer Weibchen mit ausdifferenzierten Eiern, — schwankte in

den einzelnen Versuchsjahren ganz erheblich, obschon die betreffende Pflanzenart oder -sorte 2 bzw. 3 Jahre auf der gleichen Parzelle angebaut wurde. Bei genauer Betrachtung ließen sich diese Wirtspflanzen in annähernd gleichsinnig reagierende Gruppen einordnen, wobei die unterschiedliche Befallsintensität der einzelnen Versuchsjahre gewisse Zusammenhänge mit dem jeweiligen Witterungsgeschehen der Monate März bis Juni wahrscheinlich macht.

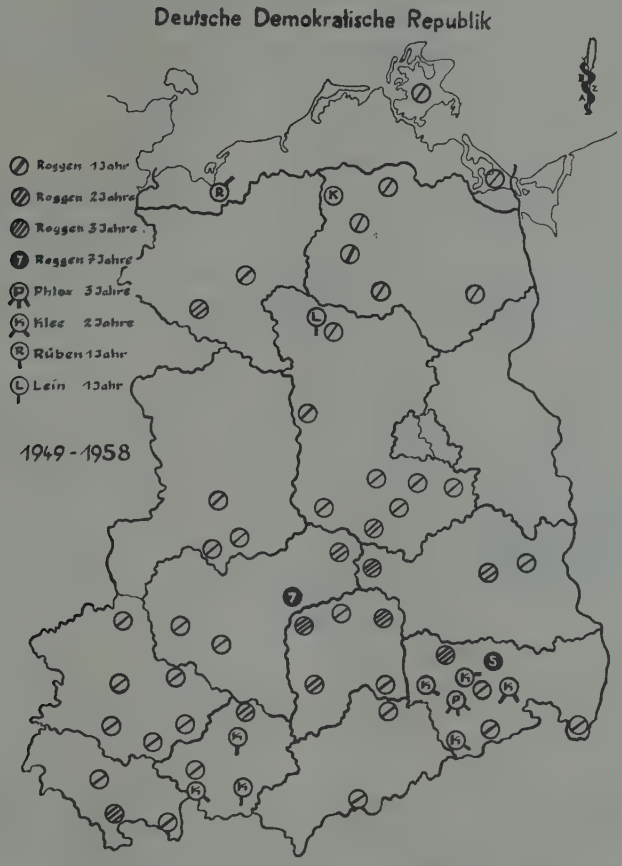

Abb. 1. Verteilung und Häufigkeit von Stockälchenschäden 1949-58.

Selbstverständlich können die allgemeinen Klimadaten einer nahegelegenen meteorologischen Station nur mit gewissen Vorbehalten für das auf der Versuchsfläche tatsächlich vorhandene Mikroklima herangezogen werden; — eine Bewertung der hier vor allem interessierenden Witterungstendenzen gestatten sie ohne Zweifel.

In der Literatur werden derartige Zusammenhänge zwischen Witterungsablauf und Stockälchenschäden verschiedentlich erwähnt. GOFFART (1941) beobachtete stärkeres Schadauftreten von *Ditylenchus dipsaci* in Jahren mit niederschlagsreichem Frühjahr; nach CAIRASCHI (1953) waren im elsässischen Tabakbau die Stockälchenschäden bei starken Regenfällen im Juni/Juli besonders ausgeprägt. DUNNING (1954) bezeichnete bei Untersuchungen an *Beta*-Rüben kühles und feuchtes Wetter als für die Nematodenentwicklung günstig. NOLTE (1957) stellte eine Korrelation zwischen erhöhten Schädigungen von Zwiebeln bei unterdurchschnittlichen Temperaturen und übernormalen Niederschlägen in den Monaten April bis Juni fest.

ERGEBNISSE

Die eigenen Ergebnisse bestätigen z.T. die Literaturangaben, jedoch reagieren die einzelnen Pflanzenarten entsprechend ihren spezifischen Ansprüchen für eine normale Jugendentwicklung durchaus unterschiedlich. Zur Verdeutlichung dieser Feststellung werden in Abb. 2 typische Vertreter von Gruppen mit eigener Verhaltensweise miteinander verglichen und zwar für die Gruppe der Hauptwirte:

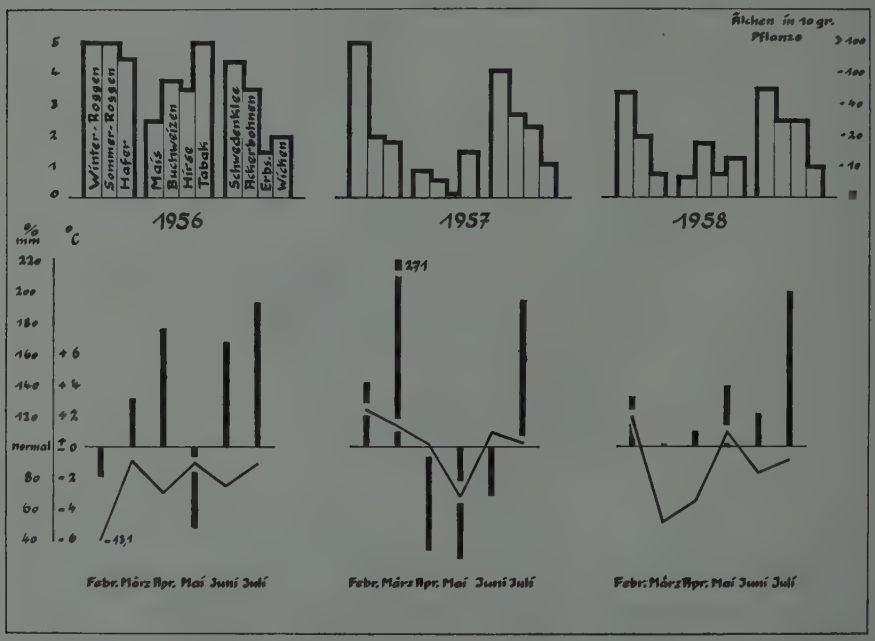

Abb. 2. Beziehungen zwischen Befallsintensität und Witterungsgeschehen bei verschiedenen Wirtspflanzengruppen 1956-58.

Winterroggen	*Secale cereale* L.
Sommerroggen	„ „
Hafer	*Avena sativa* L.

für die Gruppe der Wirte mit höheren Wärmeansprüchen:

Mais	*Zea mays* L.
Buchweizen	*Fagopyrum sagittatum* Gillib.
Hirse	*Panicum miliaceum* L.
Tabak	*Nicotiana tabacum* L.

fur die Gruppe der Wirte mit ziemlich gleicher Befallsstärke (Leguminosen):

Schwedenklee	*Trifolium hybridum* L.
Ackerbohne	*Vicia faba* L.
Speiseerbse	*Pisum sativum* L.
Sommerwicke	*Vicia sativa* L.

1956 lagen die Durchschnittstemperaturen innerhalb der interessierenden Zeitstrecke erheblich unter der Norm; die Niederschläge — mit Ausnahme des Monates Mai — darüber.

1957 war der März zu warm und wesentlich zu feucht, die Monate April bis Juni bei etwas zu kühlem, Mai ausgesprochen trocken.

1958 waren bei etwa normaler, leicht ansteigender, für den Pflanzenwuchs günstigen Regenversorgung die mittleren Temperaturen der Monate März/April wesentlich zu niedrig, der Mai zu warm, während Juni und Juli die Temperaturnorm nicht ganz erreichten.

Bei der Gruppe der Hauptwirte waren 1956 entsprechend dem Witterungsablauf und den Erfahrungen anderer Autoren Befall und Schadausmaß sehr groß; 1957 ließ es der fast an kontinentale Bedingungen erinnernde trockene Vorsommer bei Sommerroggen und Hafer nicht zu starkem Befall kommen. 1958 ermöglichte der große Temperatursprung April/Mai bei ausreichender Feuchtigkeit eine sehr schnelle Jugendentwicklung, so daß — trotz 3 jährigem ständigen Anbau einer Hauptwirtspflanze bei Winterroggen und Hafer die Befallsintensität deutlich geringer als im Vorjahr war.

In der Gruppe der Wirtspflanzen mit höheren Wärmeansprüchen — Aussaattermin für Mais, Buchweizen u. Hirse um den 15.5., für Tabak etwa der 25.5. — läßt sich die geringe Befallsstärke des Jahres 1957 mit den über der Norm liegenden Junitemperaturen bei zu geringen Regenmengen, d.h. diesen Pflanzen mehr zusagenden kontinentalen Bedingungen erklären; das unterstreicht die höhere Infektionsquote bei zu kühlem und etwas zu feuchtem Juni im Jahre 1958. Allerdings konnten die von Cairaschi (1953) gemachten Beobachtungen — stärkere Schäden an Tabak bei übernormalen Niederschlägen im Juni/Juli — im Jahre 1958 bei zu warmem Mai nicht bestätigt werden. Der wesentlich zu kühle und zu feuchte Vorsommer des Jahres 1956 und die dadurch ausgelöste Befallsintensität bei den Pflanzen dieser Gruppe spricht für sich selbst.

Die 3. Gruppe, Wirte mit verhältnismäßig gleichbleibender Befallsstärke, enthält Pflanzenarten, die bei teilweise großer ökologischer Streubreite — vor allem im Ackerfutterbau — ursprünglich feucht-kühle Standorte des maritimen Klimas bevorzugten. Hier ist ein Einfluß der Jahreswitterung nicht so deutlich nachzuweisen. Die nur wenig schwankenden Befallswerte lassen aber den Schluß zu, daß die in den anderen, bisher erwähnten Gruppen konstatierte variable Befallsintensität mehr auf das witterungsbedingte, unterschiedliche Pflanzenwachstum als auf eine Beeinflussung der Nematodenaktivität zurückgeführt werden muß. Deutlich kommt diese Feststellung bei den verschiedenen, 1957 und 1958 untersuchten Winterroggensorten zum Ausdruck (Abb. 3). Nach mittleren Monatstempera-

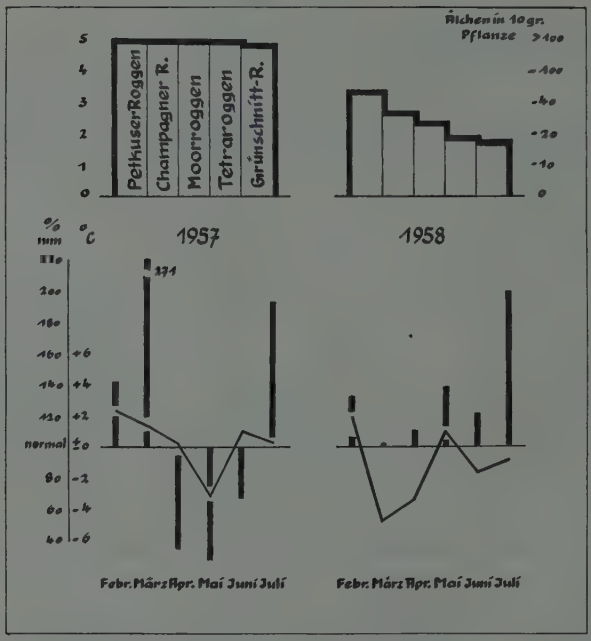

Abb. 3. Befallsintensität und Witterungsgeschehen bei verschiedenen Roggensorten 1957-58.

turen von —1.7° C im März 1958 (= 4.7° C unter der Norm) und + 4.7° C im April (= 3.4° C unter der Norm) sprang das Monatsmittel für Mai auf 14.5° C (= 1.0° C über der Norm) bei gleichzeitig um etwa 40 % erhöhter Niederschlagsmenge. Dieser Witterungsablauf führte zu so schlagartigem Wachstum und so schnellem Schossen daß die Älchen nur eine schwache Population aufbauen konnten. Bei den ersten Untersuchungen Anfang bis Mitte Mai waren mit Ausnahme des Petkuser Roggens in den Pflanzen kaum Stockälchen nachzuweisen. Ferner lag bei einigen mehrjährigen Gräsern:

Glatthafer	*Arrhenatherum elatius* (L.) Presl.
Wehrlose Trespe	*Bromus inermis* Leysser
Knaulgras	*Dactylis glomerata* L.
Schafschwingel	*Festuca ovina* L.
Wiesenschwingel	*Festuca pratensis* Huds.
Welsches Weidelgras	*Lolium multiflorum* Lamk.
Wiesenlieschgras	*Phleum pratense* L.

die Befallsstärke in den Aussaatjahren wesentlich höher als im zweiten Anbaujahr. Dieses Verhalten kann als eine gewisse Altersresistenz oder höhere Jugendanfälligkeit gedeutet werden.

In einigen anderen, im Sortiment angebauten einjährigen Kulturpflanzenarten wurden nicht in allen Jahren — trotz mehrfacher, zeitlich gestaffelter Untersuchungen — reife Weibchen mit ausdifferenzierten Eiern gefunden; offensichtlich bieten sie *Ditylenchus dipsaci* in Abhängigkeit von Umweltfaktoren nicht in jedem Jahr die Möglichkeit zur vollen Reproduktion.

Als Beispiele können genannt werden:

Zwiebel	*Allium cepa* L.
Hanf	*Cannabis sativa* L.
Mohn	*Papaver somniferum* L.
Winterrübsen	*Brassica rapa oleifera* L.
Stoppelrübe	*Brassica rapa rapifera* L.
Gelbe Lupine	*Lupinus luteus* L.
Serradella	*Ornithopus sativus* Brot.
Futtermöhre	*Daucus sativus* Hoffm.
Sonnenblume	*Helianthus annuus* L.
Topinambur	*Helianthus tuberosus* L.

Taxonomisch ist eine sichere Unterscheidung der sogenannten „biologischen Rassen" von *Ditylenchus dipsaci* bisher nicht durchzuführen. Die Aggressivität der einzelnen Stockälchen-Herkünfte gegenüber bestimmten Kulturpflanzen ist praktisch die einzige Möglichkeit, sie in vorhandene „Rassen" einzuordnen, bzw. eine neue Rasse abzugrenzen; ein Verfahren wie es SEINHORST (1957) auf dem IV. Internationalen Symposium über Nematologie im Hamburg erwähnte. Meist stützt man sich dabei auf kurzfristige Versuche im Freiland — wobei oft nur der sichtbare Schaden als Kriterium für die Einstufung als Wirtspflanze gilt — oder auf großenteils mit künstlichen Infektionen durchgeführte Gewächshausversuche.

Bei einjährigen Erhebungen wird man infolge der aus den vorstehend geschilderten eigenen Arbeiten abzuleitenden Variabilität der Befallsstärke zu unterschiedlichen Ergebnissen kommen können. Andererseits werden die unter Gewächshausbedingungen erzielten Befunde auf Grund der abweichenden Umweltgegebenheiten den Relationen im Freiland nicht immer Rechnung tragen und daher nur eine bedingte Aussagekraft besitzen. So hatte MARCUS (1954) zwar an Augenstecklingen der Kartoffelsorten „Erstling", „Bona" und „Ackersegen" unter

Gewächshausbedingungen starke, durch *Ditylenchus dipsaci* verursachte Schäden und Deformationen beobachtet, im Feldbestand trat die Erscheinung nur verschwindend gering auf.

Es drängt sich die Frage auf, ob nicht in manchen Fällen die Abgrenzung einer neuen „biologischen Rasse" lediglich auf der willkürlichen Auswahl des Testsortimentes und den jeweils vorliegenden spezifischen Umweltfaktoren beruht; daher streng genommen nur eine mögliche Modifikation innerhalb der gegebenen Variationsbreite darstellt.

Von den 1956-58 angebauten Kulturpflanzen wurden — soweit technisch durchführbar — Samen geerntet und über Winter auf etwaige Übertragungsmöglichkeiten für Stockälchen untersucht (Tabelle I). Nach den Befunden muß

Tabelle I

Samenuntersuchung auf Stockälchenbesatz 1956-58

		1956	1957	1958
Avena sativa	Hafer	0	0	0
Hordeum sativum L.	Wintergerste	0	0	0
	Sommergerste	—	0	0
Panicum miliaceum L.	Rispenhirse	—	0	0
Secale cereale L.	Petkuser Winterrogen	0	0	0
	Champagner Roggen	—	0	0
	Tetraroggen	—	0	0
	Moorroggen	—	0	0
	Grünschnittroggen	—	0	0
	Petkuser Sommerroggen	—	0	0
Triticum aestivum L.	Winterweizen	0	0	0
	Sommerweizen	—	0	0
Zea mays L.	Mais	—	0	—
Cannabis sativa L.	Hanf	—	0	—
Fagopyrum sagittatum Gillib.	**Buchweizen**	+	+	+
Spergula arvensis L.	**Spörgel**	+	0	0
Papaver somniferum L.	Mohn	0	0	0
Brassica napus oleifera L.	Winterraps	0	0	0
Brassica rapa oleifera L.	Winterrübsen	0	—	0
Sinapis alba L.	weißer Senf	—	0	—
Lupinus luteus L.	gelbe Lupine	—	0	—
Melilotus albus Med.	Steinklee	—	0	—
Pisum arvense L.	Felderbse	—	0	—
Pisum sativum L.	Speiseerbse	0	0	0
Trifolium incarnatum L.	Inkarnatklee	—	0	—
Trifolium pratense L.	Rotklee	—	0	—
Vicia faba L.	**Ackerbohne**	+	0	0
Vicia sativa L.	**Sommerwicke**	+	+	—
Vicia villosa Roth.	Zottelwicke	0	0	0
Linum usitatissimum L.	Ölfaserlein	0	0	—
	Faserlein	0	0	—

0 = keine *D. dipsaci* festgestellt
\+ = *D. dipsaci* festgestellt
— = Keine Untersuchung

zumindest bei Buchweizen (*Fagopyrum sagittatum*), Spörgel (*Spergula arvensis*), Ackerbohne (*Vicia faba*) und Sommerwicken (*Vicia sativa*) mit Samenübertragung gerechnet werden. Für Ackerbohnen waren DIERCKS & KLEWITZ (1957) zu gleichen Feststellungen gekommen.

Beim Vergleich der in den Versuchsjahren 1955-1958 ermittelten Wirtspflanzen — dabei gelten als „Wirtspflanze" alle untersuchten Pflanzenarten, bei denen mindestens in einem Jahr adulte Weibchen mit ausdifferenzierten Eiern gefunden wurden — mit der von GOODEY herausgegebenen Wirtspflanzenliste bleiben einige Pflanzenarten ungenannt. Sie sind in Tabelle II zusammengestellt.

TABELLE II

In der Wirtspflanzenliste von GOODEY-FRANKLIN *1956 nicht genannte Wirte von* Ditylenchus dipsaci (*Ergebnisse Weißbach 1955-1958*)

Equisetum arvense L.	Ackerschachtelhalm
Bromus inermis Leysser	**Wehrlose Trespe**
Scleranthus annuus L.	Ackerknäuel
Lupinus angustifolius L.	**bl ue Lupine**
Ornithopus sativus Brot.	**Serradella**
Vicia cracca L.	Vogelwicke
Erodium cicutarium L'Hér.	Reiherschnabel
Viola tricolor L.	Sandstiefmütterchen
Phacelia tanacetifolia Benth.	**Phacelia**
Myosotis discolor Pers.	Ackervergißmeinnicht
Galeopsis ladanum L.	Ackerhohlzahn
Melampyrum arvense L.	Ackerwachtelweizen
Veronica spicata L.	ähriger Ehrenpreis
Helianthus annuus L.	**Sonnenblume**
Helianthus tuberosus L.	**Topinambur**

Für den Phytopathologen ist die von Umweltfaktoren abhängende, d.h. ökologisch gesteuerte Dynamik in den Beziehungen zwischen Wirt und Parasit nichts Neues oder Ungewöhnliches — vor allem bei pilzlichen Pathogenen. Es ist vielleicht angebracht, die Relativität des Begriffes „Wirtspflanze" auch im Rahmen nematologisch-taxonomischer Untersuchungen mit zu berücksichtigen.

SUMMARY

Many years' investigations on host plants of stem eelworm (Ditylenchus dipsaci)

Field experiments between 1956 and 1958 on the host range of a race of *Ditylenchus dipsaci* attacking rye, agree with published data in showing a probable connection between intensity of attack and weather in the period March-June. The behaviour of individual species, however, is different under similar weather conditions and is determined by their specific ability for rapid early development. Since the intensity of attack and the production of symptoms is modified by environmental conditions to a considerable extent, it is easy to suppose that many cases of the deliniation of so-called biological races rests solely on choice of test material and on the environmental conditions applied. This is true especially for tests of short duration made under glasshouse or laboratory conditions. Genetically speaking distinct biological races may not be concerned but only possible modifications from the given range of variation. In addition data on seed transmission and previously unknown host plants are included.

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ÜBER EIN VERMUTLICH PFLANZENSCHÄDIGENDES AUFTRETEN EINES FREILEBENDEN NEMATODEN, *LONGIDORUS MAXIMUS* (BÜTSCHLI) AN EINER REIHE VON KULTURPFLANZEN

VON

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Wenn man die große Zahl von Arbeiten überblickt, die sich mit Pflanzenschäden durch freilebende Nematoden befassen, so fällt auf, daß die weitaus größte Zahl der bekannten Arten und Gattungen bei den Tylenchida und nur eine geringe Zahl bei den Dorylaimoidea einzuordnen sind. Es mag deshalb von Interesse sein, über Pflanzenschäden in Bayern zu berichten, die mit großer Wahrscheinlichkeit auf einen Vertreter dieser Gruppe zurückzuführen sind, der von verschiedener Seite übereinstimmend als *Longidorus maximus* (Bütschli 1874) Thorne & Swanger 1936 bestimmt wurde.

In der Literatur wird *L. maximus* nur zweimal genannt. Zuerst von BÜTSCHLI (1874), der diese Nematodenart allerdings nur in einem Exemplar in einer Gartenerde bei Kiel fand und sie als *Dorylaimus maximus* beschrieben hat und von FRANCÉ (1959, frühere Ausgabe 1923), der, anläßlich von Nematoden-Beobachtungen in den Jahren 1917-1919 berichtet, daß diese Art in Waldhumus, vor allem aber auch in Gartenerde in größerer Menge vorkam. Eine pflanzenschädigende Wirkung wird jedoch nicht erwähnt.

VERBREITUNG UND ART DER SCHÄDEN

Die ersten mit starken Wachstumshemmungen verbundenen Schäden, die mir bekannt wurden und die auf die Einwirkung freilebender Nematoden hindeuteten, traten 1958 in einer großen Gladiolen-Kultur einer Blumengärtnerei in Forchheim (Oberfranken) in Form von einzelnen kleinen Herden auf. Noch im gleichen Jahre wurden ähnliche Pflanzenschäden von verschiedenen anderen Stellen gemeldet, die Ende Juli eingehend untersucht wurden. Sie fanden sich im Gemüsebaugebiet bei Bamberg a. Main und zwar an Möhren (*Daucus carota* L.), Salat (*Lactuca sativa* L.), Schwarzwurzeln (*Scorzonera hispanica* L.), Sellerie (*Apium graveolens* L.), Gurken (*Cucumis sativus* L.), Lauch (*Allium porrum* L.) und Zwiebeln (*Allium cepa* L.). Ob die Schäden, die an Weißkohl (*Brassica capitata* L.) beobachtet wurden, ebenfalls die gleiche Ursache hatten, bleibt fraglich. Auch Franzosenkraut (*Galinsoga quadriradiata* R. u. Pav.), Vogelmiere (*Stellaria media* (L.) Cyrillo), Gänsefuß (*Chenopodium album* L.) und Ackergauchheil

(*Anagallis arvensis* L.) waren stark im Wuchs gehemmt. Nach den Aussagen der Gärtner sollen auch Spinat (*Spinacea oleracea* L.) und Stiefmütterchen (*Viola tricolor* L.), nicht aber Rettich (*Rhaphanus sativus* L.) in ähnlicher Weise geschädigt werden.

Die gleichen Schäden an Zwiebeln wurden in der Nähe von Nürnberg und an Zwiebeln, Salat und Sellerie in einem Gemüseanbaugebiet bei Kitzingen a. Main festgestellt. Ferner wurden stärkere Schäden an Tabak (*Nicotiana tabacum* L.) auf zwei Feldern in der Nähe von Schwabach bei Nürnberg und weiter solche an Runkelrüben (*Beta vulgaris* L.), Sonnenblumen (*Helianthus annuus* L.) und Kartoffeln (*Solanum tuberosum* L.) bei Scheinfeld (zwischen Nürnberg und Würzburg) beobachtet.

Da diese Schadbilder an diejenigen erinnerten, die bei Sämlingen von Fichten (*Picea excelsa* Link.) und Kiefern (*Pinus silvestris* L.) bereits früher in Baumschulen bei Miltenberg im Maintal aufgetreten waren, wurden auch hier eingehende Untersuchungen der sich in der Zwischenzeit noch wesentlich vergrößerten Schadherde vorgenommen. Über diese Schäden hat bereits Weischer (1956) berichtet. Neu hinzugekommen waren hier insbesondere starke Schäden an Sämlingen von Weiß- und Roterlen (*Alnus incana* DC. und A. *glutinosa* Gaertn.) (Abb. 1).

An allen beobachteten Schadstellen die sich als größere oder kleinere Herde von kreisartiger, ovaler oder unregelmäßig gezeichneter Form bemerkbar machten, zeigten die Pflanzen eine starke Wuchshemmung. Bei stärkerer Schädigung waren sie selbst nach längerem Wachstum nur wenige cm hoch geworden. Sie hatten, wie z.B. der Tabak, die Runkelrüben oder die Schwarzwurzeln wenige kleine Blätter mit z.T. vergilbten Spitzen oder waren, wie z.B. die Fichten- und Kiefernsämlinge, unter Braun- oder Braunrotverfärbung ganz abgestorben. In einigen Schwarzwurzelfeldern und auch in den Baumschulen hatten die Schäden einen solchen Umfang angenommen, daß praktisch das ganze Feld bis auf wenige Restflächen mit solchen Kümmerpflanzen bestanden war. Die Möhren bildeten ebenso wie die Runkelrüben nur einen äußerst dünnen Wurzelkörper aus.

Auch das gesamte Wurzelwerk zeigte eine starke Wachstumshemmung. Die Wurzeln waren meist verkürzt, die Spitzen angeschwollen und hakenförmig gekrümmt (Abb. 2). Auch die längeren Wurzeln zeigten vielfach Veränderungen ihrer Spitzen. Im jugendlichen Stadium waren sie weiß bis gelblichweiß; später wurden sie braun und konnten schließlich ganz absterben. In manchen Fällen erfolgte auch ein Weiterwachsen der Wurzelspitzen, so daß die Verdickungen etwas hinter die Spitze zu liegen kamen. Besonders ausgeprägt sind die „Hakenwurzeln" an Zwiebeln, Lauch und Sellerie. Durch die Wurzelverkürzungen und -verdickungen und das schließliche Braunwerden und Absterben nimmt das ganze Wurzelwerk bei manchen Pflanzenarten ein eigenartiges struppiges Aussehen an.

Im Wurzelbereich der erkrankten Pflanzen wurden fast stets mehr oder weniger Individuen von *L. maximus* festgestellt, während sie an den gesunden Pflanzen im allgemeinen nicht oder doch nur in einzelnen Exemplaren zu finden waren.

F. SPRAU: *Longidorus maximus an Kulturpflanzen.*

Abb. 1. Nematodenschäden an Roterlen (*Alnus spp.*) in einer Baumschule.

Abb. 2. Die stark verdickten, hellen und hakenförmig gekrümmten Wurzelenden einer Selleriepflanze (*Apium graveolens* L.) aus einer Nematodenschadstelle.

F. SPRAU: *Longidorus maximus an Kulturpflanzen.*

Abb. 4. Bekämpfungsversuch mit Shell-DD bei Tabak. Im Hintergrund behandelt, im Vordergrund unbehandelt.

Das stärkste Auftreten war an den Runkelrüben bei Scheinfeld zu beobachten, wo sich die Tiere dicht gehäuft unmittelbar an den Wurzeln fanden.

Eine Untersuchung der Wurzeln und Herde ergab wohl das Vorhandensein von verschiedenen anderen pflanzenparasitären Nematodenarten; diese waren aber zahlenmäßig so gering, daß sie als Ursache des Schadens wahrscheinlich nicht in Betracht kommen.

Wie eine Umfrage bei den Gärtnern der Bamberger Gemüsebaubetriebe ergab, waren die Schäden schon seit mehreren Jahren bekannt und nahmen immer mehr an Umfang zu. Man hatte auch bereits an den Wurzeln, die als „Würmer" bezeichneten Nematoden festgestellt und als Ursache für die Schäden vermutet. Nach den Angaben der Gärtner und unseren bisherigen Beobachtungen treten diese Schäden nur in den Sommermonaten, nicht dagegen im zeitigen Frühjahr und Herbst auf. In manchen Fällen haben sie sich bis zu einem gewissen Grade im Herbst wieder verwachsen. Ferner scheinen sie nur an jungen Pflanzen, also an Sämlingen (Schwarzwurzeln, Kiefern, Fichten) oder an Setzlingen und Stecklingen (Zwiebeln, Salat, Sellerie, Rebenstecklingen) aufzutreten. Dies hat wahrscheinlich seine Ursache darin, daß vor allem die neu entstehenden oder noch sehr jungen Wurzeln von den Nematoden geschädigt werden. Die eigenartigen Verdickungen und Hakenbildungen der Wurzelspitzen werden wohl dadurch verursacht, daß das Saugen der Nematoden in der Wachstumszone der Wurzeln, also unmittelbar hinter der Spitze erfolgt und das empfindliche meristematische Gewebe in der geschilderten Weise reagiert. Die Länge, die die Wurzel erreicht, dürfte vom Zeitpunkt der Wurzelentwicklung abhängen, zu dem der Angriff der Nematoden stattfindet.

Im Jahre 1959 traten die Schäden im wesentlichen an den gleichen Stellen wie im vergangenen Jahr auf und waren stets an das Vorhandensein van *L. maximus* gebunden. Sie wurden jedoch z.T. durch Trockenheitsschäden überlagert. Ein neues Auftreten wurde in einer Gärtnerei in der Nähe von Schwabach bei Nürnberg, also in der Nähe der Tabakschäden und im Schwemmlandgebiet des Mains, nördlich von dem bereits erwähnten Gebiet bei Kitzingen und südlich Würzburg in Rebschulen festgestellt. Während die Schäden in den Rebschulen an einjährigen, auf Amerikaner-Unterlage veredelten Rebstecklingen (*Vitis vinifera* L.) auftraten, konnten in der Gärtnerei, außer an den bereits bekannten Pflanzen, Schäden an Chrysanthemen (*Chrysanthemum indicum* L.), Bohnenkraut (*Satureja hortensis* L.) Petersilie (*Petroselinum sativum* Hoffm.), Schnittlauch (*Allium schoenoprasum* L.) und Dahlien (*Dahlia variabilis* (Willd.) Desf.) beobachtet werden. Auch hier wurde vom Besitzer der Gärtnerei in Übereinstimmung mit den Berichten der Bamberger Gärtner versichert, daß die Schäden bei Stiefmütterchen besonders stark seien.

DIE ABHÄNGIGKEIT DES AUFTRETENS DER SCHÄDEN UND DER NEMATODEN VON DEN ÄUSSEREN BEDINGUNGEN

Böden, die ein gehäuftes Vorkommen von *L. maximus* in Verbindung mit Schäden zeigten, hatten einen pH-Wert von 5,6-6,8. Ferner scheint das Auftreten der Nematoden an sandige bis feinsandig-lehmige, im allgemeinen humusreiche Böden, mit guter Durchlüftung gebunden zu sein. Auf benachbarten schwereren Böden wurden weder *L. maximus* noch Schäden beobachtet. Weiter fällt auf, daß eine Reihe der Schäden gerade in den Schwemmsandböden des Maintales und seiner Nebenflüsse auftritt (Abb. 3). Die Böden sind infolge des ziemlich hohen

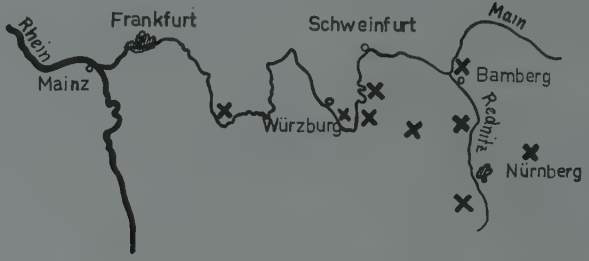

Abb. 3. Bis zum Jahre 1959 festgestellte Schadstellen an Pflanzen (= X), in denen zugleich *L. maximus* gefunden wurde.

Grundwasserstandes auch bei Trockenheit verhältnismäßig feucht. Neben der guten Durchlüftung scheint also auch eine gewisse stetige Feuchtigkeit für die Entstehung der Schäden und die Entwicklung des Nematoden eine Voraussetzung zu sein. Daß das tatsächlich sogar in hohem Maße der Fall ist, hat gerade der diesjährige trockene Sommer gelehrt. Wo außerdem, wie z.B. in den Rebschulen, künstlich beregnet oder bewässert wurde, waren die Schäden besonders stark.

Weiter ist auch die Temperatur für das Auftreten der Schäden und des Nematoden ein ganz wesentlicher Faktor. Das wird besonders dadurch bestätigt, daß diese bis jetzt hauptsächlich in den wärmeren Gebieten des Maintales aufgetreten sind und nur in den warmen Sommermonaten sich anscheinend eine Population aufbaut, die u.U. zu schädigen vermag. Auch von den Bamberger Gärtnern wurde beobachtet, daß die Schäden bei warmer Witterung stärker waren und dann auch dort auftraten, wo sonst keine festzustellen waren.

In bezug auf die Wirkung der Bodenbearbeitung und der Vorfrucht auf das Auftreten der Schäden liegen erst wenig Erfahrungen vor. In dieser Hinsicht ist jedoch das Baumschulgebiet von Miltenberg interessant, weil sich hier die Schäden sowohl auf den Flächen einstellen, die seit längerer Zeit ackerbaulich und gärtnerisch genutzt wurden, als auch auf solchen, die als Wiese umgebrochen worden waren. Dies gibt einen Hinweis dafür, daß auch auf frischen Wiesenumbrüchen in bestimmten Lagen immer mit Ausfällen gerechnet werden muß.

BEKÄMPFUNGSMASSNAHMEN

Schon 1957 waren von der Staatl. Obst- und Gartenbaustelle in Bamberg auf Schadstellen im Bamberger Gemüsebaugebiet mit Vapam, das entsprechend den Vorschriften angewandt worden war, Bekämpfungsversuche mit gutem Ergebnis durchgeführt worden. Im gleichen Jahr wurden auch von WAGNER (1959) erfolgreiche Bekämpfungsversuche mit Shell-DD und Vapam in einer Baumschule im unteren Maintal bei Kiefersämmlingen durchgeführt. 1959 führten wir auf Schadstellen in den beiden oben erwähnten Tabaksfeldern und dem Runkelrübenfeld mit den gleichen Mitteln Bekämpungsversuche durch. Vapam wurde in allen Versuchen mit 60 ccm/qm, DD-Shell in einer Menge von 60 und 100 ccm/qm angewandt. Während auf dem einen Feld weder bei Frühjahrs- noch bei Herbstbehandlung ein Erfolg zu sehen war — auf allen Parzellen standen die Tabakpflanzen gleich gut und zeigten keine Schäden —, traten auf dem anderen Feld, das nur im Frühjahr behandelt worden war, deutliche Unterschiede zutage (Abb. 4). Allerdings sind die Ergebnisse insofern eigenartig, als auf den mit DD-Shell behandelten Parzellen die Pflanzen ausgezeichnet standen, auf den Vapam-parzellen jedoch eine nicht wesentlich bessere Entwicklung zeigten als auf den unbehandelten Flächen.

Auf dem Runkelrübenfeld, wo Runkelrüben, Zuckerrüben, Mais und Zwiebeln angebaut wurden (Scheinfeld), sind die Ergebnisse, die zweifellos positiv sind, nicht leicht zu deuten. Während der Mais in keiner Weise reagiert hat, d.h. die Pflanzen waren überall ziemlich gleichmäßig gut entwickelt, standen die Zwiebeln auf der unbehandelten Parzelle sehr schlecht, auf der mit Vapam behandelten Fläche nicht viel besser; sie zeigten jedoch einen sehr guten Wuchs bei einfacher und erhöhter Gabe von DD-Shell. Die Rüben, bei denen sich nicht so große Wachstumsunterschiede ergaben, verhielten sich jedoch eher umgekehrt. Inwieweit hier ein besserer Aufschluß des Bodens oder sonstige noch unbekannte Faktoren bei der Mittelbehandlung, evtl. auch der ungewöhnliche Sommer, mitgewirkt haben, konnte nicht festgestellt werden.

Enttäuschend waren die Ergebnisse, die mit dem Anbau von *Tagetes erecta* L. und *T. parva* L. gewonnen wurden (OOSTENBRINK, KUIPER & S'JACOB 1957). Dies ist dadurch erklärlich, daß auch an den *Tagetes*-Pflanzen z.T. Wurzelverbildungen und im Wurzelraum *L. maximus* gefunden wurde.

SCHLUSSBESPRECHUNG

In der gesamten Literatur gibt es, was auch durch eine briefliche Mitteilung von Dr. OOSTENBRINK, Wageningen, bestätigt wurde, nirgends einen Hinweis, daß *L. maximus* pflanzenschädigend auftritt. Wenn man jedoch die geschilderten Beobachtungen und die Angaben aus der Literatur über verwandte Arten überprüft und vergleicht, — vor allem ist die große Ähnlichkeit der durch die Gattung *Xiphinema* hervorgerufenen Veränderungen der Wurzeln, mit den von uns

beobachteten Wurzelbildern überraschend (SCHINDLER 1957) — so gewinnt man den Eindruck, daß bei den festgestellten Schäden dieser Nematode als Ursache oder zumindest mit als Ursache in Betracht gezogen werden muß.

Für eine Beteiligung von *L. maximus* an den Pflanzenschäden spricht zunächst die Tatsache, daß in einer Reihe von Fällen die Wurzeln der geschädigten Pflanzen z.T. in außerordentlich hohem Maße von dieser Nematodenart besetzt waren, während an den Wurzeln der gesunden Pflanzen keine und im Wurzelballen nur vereinzelte Tiere beobachtet wurden. Weiter ist anzuführen, daß an räumlich weit voneinander getrennten Schadstellen stets dieser Nematode gefunden wurde, ferner, daß das damit verbundene Schadbild, vor allem an den Wurzeln, in den Grundzügen fast immer das gleiche war.

Eine Untersuchung des Wurzelraums der Pflanzen in den Schadstellen hat zwar auch das Vorkommen anderer pflanzenschädigender Nematodenarten ergeben, die jedoch meistens in geringer Zahl auftraten und keineswegs immer so treue Begleiter der geschädigten Pflanzen wie *L. maximus* waren. Auch *Pratylenchus penetrans,* der nach GOFFART (1951, 1958) ebenfalls Anschwellungen an den Wurzelspitzen, jedoch nicht in der geschilderten typischen Form verursachen kann, war bis jetzt nur an wenigen Stellen gefunden worden. Allerdings konnten bei den in letzten Zeit durchgeführten Bodenuntersuchungen neben *L. maximus* an manchen Schadstellen in Gärtnereien und Baumschulen auch *Pratylenchus penetrans* bisweilen in größerer Zahl nachgewiesen werden, so daß möglicherweise die starken Schäden nicht auf *L. maximus* allein, sondern auch noch auf die Mitwirkung anderer Nematoden zurückzuführen sind. Auffallend ist allerdings der starke Wechsel des Nematodenspektrums an den verschiedenen Schadstellen, während fast stets *L. maximus,* und zwar zu bestimmten Zeiten, in größeren Mengen zugegen war.

Trotz zahlreiche Beobachtungen, die auf eine pflanzenschädigende Wirkung von *L. maximus* hindeuten, muß diese erst durch entsprechende Versuche bewiesen werden. Aus diesem Grunde und um die noch wenig bekannte Biologie des Tieres endgültig zu klären, sind neben den seit einiger Zeit laufenden Untersuchungen der Nematodenspektren der Schadstellen eine Reihe von Laboratoriums- und Gewächshausversuchen in die Wege geleitet worden. Erst auf Grund der erhaltenen Ergebnisse kann endgültig zu den Erscheinungen Stellung genommen werden. Da auch die von BÜTSCHLI (1878) an Hand eines einzigen gefundenen Tieres gegebene Beschreibung der Art einer Ergänzung bedarf, wird mein Mitarbeiter, Herr STURHAN, sich außerdem mit der Systematik befassen und in absehbarer Zeit eine eingehende Beschreibung bringen.

Ich möchte an dieser Stelle den Kollegen Dr. Goffart, Münster, Dr. Oostenbrink, Wageningen, Dr. Osche, Erlangen sowie auch Dr. Brand, München für ihre Mühe, der sie sich bei der Bestimmung von *L. maximus* unterzogen, meinen besonderen Dank aussprechen.

SUMMARY

Observations on the appearance of plant injury in a variety of crop plants, probably caused by the free-living nematode Longidorus maximus

In recent years considerable stunting of growth of various crops has caused heavy losses of yield in parts of Bavaria. The stunting is most obvious in June, July or August and is not seen in early spring or autumn. Study of roots and surrounding soil revealed that *Longidorus maximus* was frequently present and visible with the naked eye. Affected roots were usually much shortened with swollen, whitish, hooked tips. These changes are followed by the development of necrotic areas which give the root-systems of some plants a rough appearance. The following observations suggest that *Longidorus maximus* is the probable cause of the injury: — (1) The nematode is always present in numbers around injured plants and absent or in small numbers around healthy plants. (2) The injury always follows the same pattern. (3) Other species of nematode present are either not known to be injurious or are not abundant enough to account for the injury. In a few places only *Pratylenchus penetrans* also was found in large numbers. The places where *L. maximus* appeared, were widely scattered and had well aerated, sandy soils. The wide host range of *L. maximus* is remarkable as is the occurrence of plant injury in tree nurseries, market gardens and agricultural land.

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Nematologica (Suppl. II, 1960): 56-60. — E. J. Brill, Leiden.

CHOANOLAIMUS PSAMMOPHILUS J. G. DE MAN, 1880, REDISCOVERED, AND *SYNONCHIUM OBTUSUM* N. A. COBB, 1920, AS A NATURAL ENEMY OF PLANT PATHOGENS

BY

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In 1958, Dr. Dewey J. Raski, Associate Nematologist, University of California, Davis, Calif., requested the writer to make determinations of some of his prepared slides containing what appeared to be marine nemas. Among these were slides of material from about the roots of plants on sand dunes at Moss Landing, California, collected February 9, 1955. Moss Landing is an oceanic beach about 4 miles south of Watsonville. Two slides were particularly interesting as they contained choanolaims, one specimen of which, *Choanolaimus psammophilus* de Man, 1880, has not to the writer's knowledge [2]) been reported since DE MAN (1884) illustrated it as a fresh-water nema. A few minor points are here added to the original description. The remaining specimens on the two slides were determined as *Synonchium obtusum* Cobb, 1920. Several of the latter had ingested whole *Tylenchorhynchus cylindricus* Cobb, 1913, and this evidence serves to substantiate further the Choanolaimidae as carnivorous nemas. It also brings up the interesting point that, in exposed locations, normally marine nemas may adapt to terrestrial life about the roots of spermatophytes. Whether or not the reason *Choanolaimus psammophilus* has not been previously observed about plant roots is that de Man originally described it from a somewhat brackish habitat and it is normally a marine nema seems to be an open question. Workers familiar with the places de Man made his collection in Holland would be better qualified to pass judgment.

After these determinations had been made, Drs. M. W. Allen, D. J. Raski, and Armand R. Maggenti took the writer collecting in the same general area in an effort to obtain additional living material. As to *Synonchium obtusum* and *Tylenchorhynchus cylindricus* we were successful, but, unfortunately, failed to find *Choanolaimus psammophilus* again; so no studies could be made of its adaptability to various degrees of salinity.

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[1]) Supported in part by grant from National Science Foundation.

[2]) After this article was written the reference to *Choanolaimus psammophilus* in which C. Overgaard Nielsen states that he has observed this species, in sea water, feeding on other nemas (*Natura Jutlandica* 2 : 86) has been encountered. No description or illustration is given but it confirms my opinion that it is both marine and carnivorous.

Choanolaimus psammophilus de Man, 1880

Fig. 1, A-B

Oral opening surrounded by internal circle of 6 discrete labial lobes, each bearing one internal papilla, and by an external circle of 10 papillae. Amphids dispiral, 11 by 13.5 μ, 19 μ from anterior end. Cuticle with faint, though fairly widely separate, transverse grooves, and, in each such annule, a row of punctations, which become coarser laterally. Sublateral hypodermal gland pores present. Stoma 40 μ to base, with joints in mid-region.

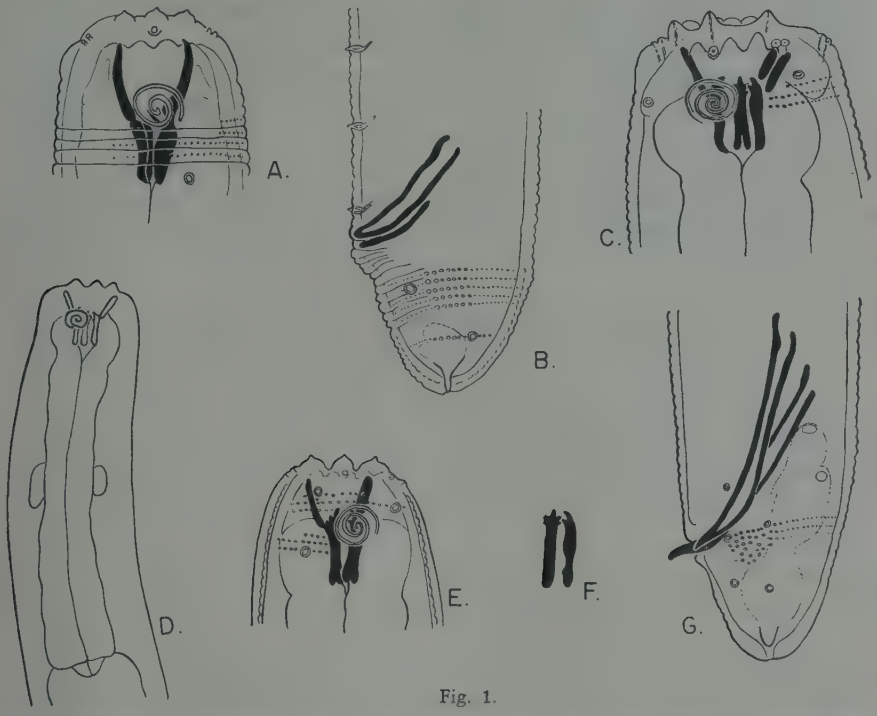

Fig. 1.

A-B, *Choanolaimus psammophilus* male: A-head, lateral view; B-tail, lateral view. C-G, *Synonchium obtusum*: C and E, head, lateral view; D, esophageal region; F, mandibles; G, male tail, lateral view.

Male. — 1.6 mm long; a, 29; b, 6.7; c, 39. Spicules 36 μ long, slightly cephalated, with little curvature. Gubernaculum parallel, simple, 20 μ long. Seven papilloid preanal supplements present, very difficult to observe. Tail 41 μ long, bluntly conoid, with inconspicuous spinneret.

Specimen. — University of California (Davis), Nematology Collection Slide No. 152.

Collector. — D. J. Raski, February 9, 1955.

Habitat and Locality. — About roots of beach spermatophytes, Moss Landing, Monterey County, California, U.S.A.

Apparently *C. psammophilus* has not yet been observed since originally collected and described by DE MAN (1880, 1884), for more recent works omit this genus and species or include de Man's illustrations and figures. The genus *Choanolaimus* is thus far, to the writer's knowledge, monospecific. De Man reported it from Dutch sand dunes, on the roots of *Galium verum* L., *Sedum acre* L., and *Hippophaes rhammoides* L., sexually mature forms being found from November to March. He gave dimensions as follows: Male 1.7 mm; a, 25; b, 7; c, 35-40; Female 2 mm; a, 25; b, 7; c, 35-50. He stated that a ventral pore and caudal glands were absent, and no papillae were observed on the male. That the presence of a spinneret and 7 preanal papilloid supplementary organs was not reported by de Man appears attributable to their both being difficult to observe. The present single specimen can hardly be used as a basis for the erection of a new species, despite these differences; and, with further study of additional material, an excretory gland and pore might well come to light.

It is conceivable that this species was originally marine and later adapted to a land habitat; and that could be the reason for its not having been reported in recent years. It certainly has not occurred in routine collections from agricultural lands made by the writer.

Synonchium obtusum N. A. COBB, 1920
Fig. 1, C-G

Original Measurements. — COBB (1920, pp. 290-291, fig. 73).

Male. — 1.02 to 1.63 mm long; a, 20-32; b, 5.5-8.9; c, 36-71. Amphids dispiral, about 9-11 μ long by 11-12.5 μ wide, 11-12 μ from anterior end. Stoma 28-37 μ deep; mandibles 18 to 20 μ long, with 3 large teeth and 2 inconspicuous smaller teeth. Cuticle finely punctate medially, coarsely punctate laterally, with paired sublateral rows of hypodermal glands. Spicules 61-67 μ long, tips bent; gubernaculum absent; testis extends forward to from 33 to 50 % of body length from anterior end. Supplementary organs not observed; tail bluntly round; spinneret terminal.

Female. — 1.26 to 1.55 mm long; a, 21-22; b, 7.0-7.4; c, 29-68; V, 54-60 %. Ovaries reflexed, extending from 12 to 18 % of body length anteriad to vulva and from 8 to 15 % posteriad; a single egg, 80 by 67 μ, in one specimen only. Mandibles 20-22 μ long. Stoma 36-37 μ deep. Amphids 9-12 μ long by 10-12 μ wide, 10-12 μ from anterior end. Excretory cell and pore not observed.

Specimens. — University of California, Plant Nematology Collection Slide No.'s 152 & 153.

Collector. — D. J. Raski, February 9, 1959.

Habitat & Locality. — About roots of spermatophytes and in sand of inner bay bottom, Moss Landing, Monterey County, California, U.S.A.

As originally described and illustrated by COBB (1920, pp. 290-291, fig. 73), the male measured 1.6 mm long; a, 32; b, 7.7; c, 50; spicules 66 μ, tips bent ventrad; testis extending to 44 % of body length from anterior end; supplements

absent; the female 1.3 mm long; a, 31; b, 6.3; c, 25; V, 61 %. Mandibles were illustrated as 22.7 μ long, amphids dispiral.

Type habitat & locality. — Seaweed, Ocean Beach, Miami, Florida, U.S.A.

GERLACH (1953, pp. 555-557, figs. 18a-e) described specimens, collected from the coastal waters of Pisa, Italy, and attributed to this species, as having the following characteristics: *Male* 2.32-2.42 mm long; a, 23; b, 6.5-9.9; c, 40.3-44.7; spicules 77-78 μ long (but, according to Gerlach's illustrations, they are only 51.6-52 μ long).

With respect to the taxonomy of these forms, the very minor differences between the measurements given by Cobb, Gerlach, and the writer might well be due to inaccuracies or to natural variation, but, as the evidence stands, this appears to be a species common to the Atlantic and the Pacific Coasts of North America as well as to the Mediterranean Sea. The only other species of *Synonchium* thus far described is *S. depressum* Gerlach, 1954 (pp. 231-232), from the coasts of Tunisia and Algeria. It differs in that the tails of the specimens are somewhat more elongate (c, 18-23.5) and the amphids only 7 μ high by 21 μ wide. Although Gerlach gives a spicular length of "77-78 μ", they are only 51.6-52 μ long according to the magnification given in his explanation of figures. His printed statement could well be a typographical error.

Ecological Comments. — Several specimens obtained from about the roots of wild spermatophytes were found, upon study, to have engorged themselves with one or more specimens of *Tylenchorhynchus cylindricus* N. A. Cobb, 1913, the latter species being a common plant parasite (pathogen) in the locality. It would appear that, in this area, we are dealing with a meeting of the marine and fresh water fauna and that *Synonchium obtusum* probably adjusts from the ocean salinity of 3.5 % total salts to a brackish or even possibly a near fresh water habitat.

ZUSAMMENFASSUNG

Choanolaimus psammophilus *J. G. de Man, 1880 wiederentdeckt und* Synonchium obtusum *N. A. Cobb, 1920, ein natürlicher Feind pflanzenpathogener Organismen*

Choanolaimus psammophilus und *Synonchium obtusum* wurden im Dünensand an Wurzeln von Spermatophyten in der Nähe von Moss Landing, Californien, gefunden und sind beschrieben. Keine dieser Arten ist vorher an der Pacifischen Küste gefunden worden. Die erste Art ist nicht mehr erwähnt worden, seitdem de Man sie beschrieben hat.

Beim Männchen des *C. psammophilus* stehen sieben Papillen vor dem After. Es gibt auch Schwanzdrüsen mit sehr kleinen Ausführungsröhren an den Hinterende. Diese mögen vorher nicht entdeckt worden sein.

Einige Exemplare des *Synonchium obtusum* enthielten im Darm *Tylenchorhynchus cylindricus*, der eine an Landpflanzen auftretende parasitäre Art ist.

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DISCUSSION

P. A. A. LOOF: The male of *Choanolaimus psammophilus* described by Dr. Chitwood differs from De Man's original description in that it possesses a spinneret and seven preanal papillae which, according to De Man, are lacking. De Man's type material is still existing. The male was found to possess a spinneret and seven small preanal papillae. Clearly these structures were overlooked at that time, and Dr. Chitwood's specimen agrees with De Man's types.

Nematologica (Suppl. II, 1960): 61-63. — E. J. Brill, Leiden.

DITYLENCHUS DIPSACI (KÜHN) AN KNOBLAUCH (*ALLIUM SATIVUM* L.)

VON

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Seit dem Jahr 1955 führen wir Beobachtungen auf einer mit *Ditylenchus dipsaci* verseuchten Fläche durch. Hauptwirtspflanzen dieser *Ditylenchus*-Population sind die *Allium*-Arten, in erster Linie die Küchenzwiebel (*Allium cepa* L.) und der Knoblauch (*Allium sativum* L.). Als weitere echte Wirtspflanzen wurden im Verlauf der mehrjährigen Beobachtungen ermittelt:

an Kulturpflanzen:

Erbse (*Pisum sativum* L.)
Buchweizen (*Fagopyrum sagittatum* L.)
Buschbohne (*Phaseolus vulgaris* L.)

und an Unkräutern:

Vogelmiere (*Stellaria media* Vill.)
Vogelknöterich (*Polygonum aviculare* L.)
Windenknöterich (*P. convolvulus* L.)
Roter Gauchheil (*Anagallis arvensis* L.)

Für diese Pflanzen konnten regelmäßig ein starker Befall durch *Ditylenchus dipsaci* festgestellt und eine Vermehrung des Nematoden in diesen Pflanzen nachgewiesen werden.

Zunächst noch unsicher ist der Wirtspflanzencharakter für folgende Kulturpflanzen:

Spinat (*Spinacia oleracea* L.)
Mohrrübe (*Daucus carota* L.)
Weißer Senf (*Sinapis alba* L.)
Futterrübe (*Beta vulgaris* L.)
Sellerie (*Apium graveolens* L.)

und für folgende Unkräuter:

Ackerkratzdistel (*Cirsium arvense* Scop.)
Hühnerhirse (*Panicum grus galli* L.)
Gemeine Quecke (*Agropyrum repens* P.B.)

Diese Pflanzen waren in der Beobachtungszeit sehr unterschiedlich befallen, eine Vermehrung der Nematoden in ihnen konnte nicht mit Sicherheit nachgewiesen werden. Für sie kann daher der Wirtspflanzencharakter zunächst nur vermutet werden; weitere Beobachtungen zur Bestätigung sind noch erforderlich.

Darüber hinaus wurden außerdem in anderen Kulturpflanzen und Unkräutern gelegentlich adulte Tiere, jedoch niemals Jugendstadien gefunden. Diese können daher nicht als Wirtspflanzen dieser Population angesprochen werden. Da ich an anderer Stelle darauf ausführlich eingegangen bin (NOLTE 1959), will ich hier auf ihre Aufzählung verzichten.

Wie schon erwähnt, wurde auf der unter unserer Kontrolle befindlichen Fläche durch die *Ditylenchus dipsaci*-Population nicht nur die Küchenzwiebel (*Allium cepa* L.), sondern auch der Knoblauch (*Allium sativum* L.) stark geschädigt. Für die Küchenzwiebel war dabei charakteristisch, daß auf den neu angesäten Flächen die Pflanzen entweder über das Zweiblattstadium gar nicht hinauskamen, sondern in diesem Stadium abstarben, oder daß nach Überwindung dieses Stadiums bzw. bei Anbau von Steckzwiebeln die für die Zwiebeln bekannten Schadbilder, Verdickungen und Krümmungen, festzustellen waren.

Für die Knoblauchpflanzen konnten die gleichen Verbildungen nicht beobachtet werden. Wir haben weder Verkrümmungen noch auffällige Verdickungen festgestellt. Oberirdisch machte sich die Schädigung durch Vergilben und Absterben der Blätter bemerkbar (Abb. 1). Bei sehr starker Schädigung der Zwiebeln verfaulten diese. In den meisten Fällen aber blieben Form und Farbe der Zwiebeln bis zur Ernte erhalten. Die befallenen Zwiebeln fühlten sich etwas schwammig an, so daß nach einiger Übung bei der Ernte die befallenen Pflanzen ohne Schwierigkeit von den nicht befallenen getrennt werden konnten. Es sei allerdings darauf hingewiesen, daß im Verlauf der Vegetationsperiode nicht alle neu gebildeten Nebenzwiebeln von den Nematoden besiedelt werden.

Das durch *Ditylenchus dipsaci* verursachte Schadbild bei Knoblauch kann sehr leicht mit dem Schadbild verwechselt werden, das durch die Zwiebelfliege (*Hylemyia antiqua* Meigen) verursacht wird. Auch in diesem Fall kommt es zum Vergilben und Absterben der oberirdischen Pflanzenteile. Die mit den Maden besetzten Zwiebeln verfaulen (Abb. 2).

Im Herbst 1958 haben wir aus dem Erntegut Knoblauchzwiebeln aussortiert, für die ein Befall aller oder wenigstens der meisten Nebenzwiebeln festgestellt werden konnte. Im Frühjahr 1959 wurden 147 solcher Nebenzwiebeln in nicht verseuchten Boden gepflanzt. Die Pflanzen liefen zunächst sämtlich auf, nach 3 Monaten waren jedoch 42,8 % der Pflanzen durch den Nematoden vernichtet worden; bis zum 3. August, d.h. nach weiteren 2 Monaten, wurde ein Ausfall von 69,3 % festgestellt. Zu dieser Zeit waren noch weitere Pflanzen deutlich geschädigt. Einige gesund gebliebene Pflanzen dürften aus Zwiebeln stammen, die nicht oder nur sehr schwach befallen gewesen waren und von vorjährigen Pflanzen stammten, deren Nebenzwiebeln nicht alle befallen waren. Dieses Ergebnis zeigt, daß im Herbst geerntete Knoblauchzwiebeln zwar den Winter überstehen,

H. W. NOLTE: *Ditylenchus dipsaci an Knoblauch.*

1 2

Abb. 1. Durch *Ditylenchus dipsaci* geschädigte Knoblauchpflanzen.
Abb. 2. Durch die Zwiebelfliege (*Hylemyia antiqua* Meigen) geschädigte Knoblauchpflanzen.

aber für eine Vermehrung nicht mehr verwendet werden können. Ausfälle durch die Zwiebelfliege waren auf dieser Versuchsfläche nicht festzustellen.

Außerdem wurden im Frühjahr auf der seit 1955 unter Kontrolle befindlichen verseuchten Fläche gesunde Knoblauchzwiebeln ausgepflanzt. Hier wurde zwar ein größerer Ausfall durch die Zwiebelfliege verursacht, der Befall dieser Zwiebeln mit *Ditylenchus dipsaci* blieb jedoch minimal. Auf dieser gleichen Fläche blieben im Frühjahr 1959 auch die Küchenzwiebeln fast befallsfrei. Dies erklärt sich daraus, daß das Frühjahr 1959 in unserem Gebiet extrem trocken war. Die Niederschlagsmengen in den Monaten April und Mai lagen wesentlich unter dem langjährigen Mittel. Damit wird bestätigt, was schon früher von SEINHORST (1950, 1956), GOFFART (1951) und mir (NOLTE 1957) festgestellt werden konnte, nämlich, daß zur Zeit des anfälligen Entwicklungsstadiums der Wirtspflanze die Bodenfeuchtigkeit hoch liegen muß. Nur dann sind für den Nematoden günstige Bedingungen vorhanden. Diese Folgerung gilt nicht nur für die Küchenzwiebel, für die wir in fünfjährigen Beobachtungen (NOLTE 1959) den Nachweis erbringen konnten, sondern läßt sich auch aus den zweijährigen Beobachtungen für den Knoblauch folgern.

SUMMARY

Ditylenchus dipsaci on garlic (*Allium sativum* L.)

The symptoms on garlic attacked by *Ditylenchus dipsaci* are: the bulb tissue is loose but not swollen and the leaves die from the tip downwards. The injury may easily be mistaken for injury by the onion fly. Hostplants of the population in question are besides onion and garlic: pea, buckwheat, dwarf bean and weeds. Rainfall in April and May affects attack by the nematode.

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CONTROL OF *PRATYLENCHUS PENETRANS* (COBB), CAUSAL AGENT OF ROOTROT IN LILIES

BY

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A rootrot of Japanese lilies (*Lilium speciosum* Thunb. v. *rubrum* Mast.) occurs in some areas of Belgium and may cause total loss of the flower crop. VAN HELL (1932) & FEEKES (1931) discovered the fungus *Cylindrocarpon radicicola* in rotting roots and considered it the cause of the disease. But MCWORTHER & ANDERSON (1951) and SLOOTWEG (1956) showed that *Pratylenchus* and *Hoplolaimus uniformis* Thorne appear to be the primary cause with the fungi *C. radicicola* and *Fusarium oxysporum* playing a secondary rôle.

Early yellowing of the crop, small necrotic lesions on growing roots and older roots decaying are the symptoms of the disease. In root samples from diseased areas a dense population of *Pratylenchus penetrans* was found and in the soil this nematode and some *Hoplolaimus sp.* occurred.

CONTROL MEASURES

Fumigation of the soil

A fumigation experiment in duplicate was started in September 1957 in an infected nursery on a sandy loam soil near Antwerp. Four litres of DD per are were applied at a depth of 20 cm, the temperature was 12° C and the water content of the soil 12 %. Population densities of various nematodes were determined in samples taken just before and 5½ month after the treatment. Counts of *Pratylenchus penetrans* and yields of lily bulbs are given in Table I.

TABLE I

Effect of soil treatment with DD on Pratylenchus penetrans *in the soil and on yield of lily bulbs*

Treatment	*Pratylenchus penetrans* per 100 ml of soil		Weight of 50 bulbs in kg	
	15.9.57 (before treatment)	31.3.58 (after treatment)	Planted with roots	Planted without roots
Untreated	75	97	2.15	2.07
DD 4 l/are	100	2	4.07	4.05

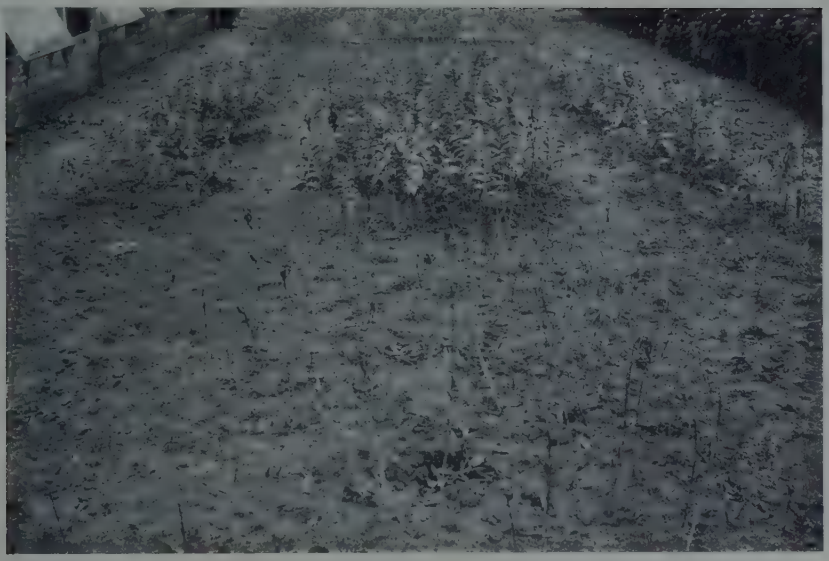

Fig. 1. — View of the crop at the end of July: (treated plot in the background).

Fig. 2. — Picture of the harvested bulbs.

To compare the effect of the treatment on the crop, each plot was planted partly with bulbs thoroughly cleaned of any remaining roots and partly with bulbs still retaining some. No difference was obtained by cleaning the bulbs (Table I). There was however a remarkable difference in growth between the fumigated plots and the controls. In the first case the crop was healthy and growth was excellent until the end of October. The control crop on the contrary died off almost completely in July (fig. 1).

The bulbs were lifted in the middle of November. Table I shows the total weight of fifty bulbs from each plot and fig. 2 a picture of the lifted bulbs. The weight of the bulbs was almost doubled by the fumigation.

Table II shows the number of *Pratylenchus* in the roots of the lifted bulbs and in 100 ml of soil after removal of the crop.

TABLE II

Number of Pratylenchus penetrans *in roots and soil of lily bulbs grown on treated and untreated plots*

Treatment	In 10 g of roots	in 100 ml of soil around roots	whole plot
Control	4972	1315	290
DD 4 1/are	497	89	15

As shown in table II, the treatment resulted in a considerable decrease of the *Pratylenchus* population in the roots and in the soil, but the remaining population is tenfold the number of eelworms estimated to be sufficient to cause rootrot disease in bulbs (SLOOTWEG, 1956).

Hot-water-treatment of the bulbs

Cleaning the bulbs of every piece of root had no effect in the fumigation experiment. Nevertheless we paid attention to the possibility of *Pratylenchus* hibernat-

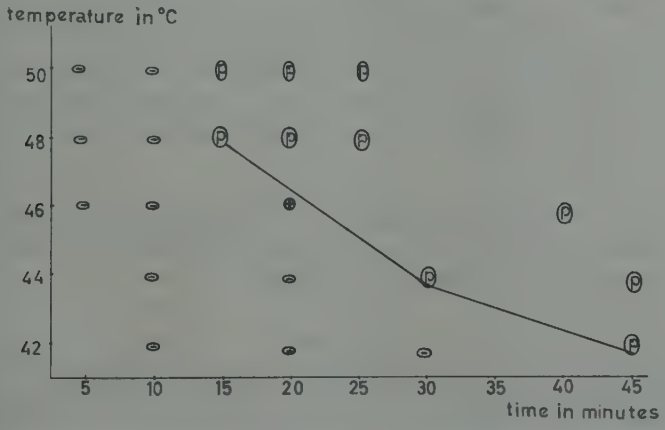

Fig. 3. — Hot-water-formalin treatment of bulbs of the Japanese lily. +: effective treatment: no nematodes; —: ineffective treatment; P: phytotoxic treatment.

ing in the roots. Our aim was to get bulbs completely free from eelworms by a hot-water-treatment (+ 0,5 % formaldehyde) of the bulbs.

Some 300 commercial bulbs of the Japanese lily from an infested field were treated in the beginning of March and then planted in flower pots of 12 cm diameter, filled with steam sterilized soil. The results of the treatments are given in fig. 3 and table III.

TABLE III

The effect of hot-water-formalin treatment on the number of Pratylenchus penetrans *in roots of Japanese lily. "P" phytotoxic effect.*

Temp. in C°	Time of treatment in min.	Number of *Pratylenchus penetrans* per bulb (4 months after planting)
42	30	10-0-1-3.
	45	6-6-6-0-8. "P"
44	20	9-12-3-19-3-0.
	30	7-3-0-7-0-1. "P"
	45	3-0-10-1-0-. "P"
46	5	53-9-21-35-3-8.
	10	4-22-0-1-3-0.
	20	0-3-0-0-0-0-0-0.
	40	0-0-0-0-0-0. "P"
48	5	7-0-1-14-7-0-2.
	10	24-0-2.
	15	P.
50	5	17-26-0-42-0-4.
	10	0-2-12-19-1-14.
	15	P.
Control		7-28-212-6-125-25-119-102-25-9.

We agree with SLOOTWEG's (1956) findings that stock bulbs with dried up roots, are not a cause of further spread of rootrot because they do not contain any *Pratylenchus*. But the results show that stock bulbs the roots of which have not been allowed to shrivel may spread the disease and this aspect warrants further investigation. However control by hot-water-treatment at 46° C for 20 minutes was efficacious.

The authors are grateful to Ir Vercammen for notifying the case near Antwerp, Prof. Ing. J. van Holder for the identification of the fungi and Mr F. G. W. Jones for help and advice in preparing the English text.

RÉSUMÉ

Les auteurs ont effectué un traitement de désinfection du sol au moyen de DD (4 l/are) dans un champ de lis du Japon (*Lilium speciosum* Thunb. var. *rubrum* Mast.), infesté par *Pratylenchus penetrans*, agent causal de la pourriture des racines. De ces recherches il ressortit que ce traitement est très efficace: dans les parcelles traitées les nématodes parasitaires furent quasi complètement détruits et le poids des bulbes récoltés y était environ le double des parcelles non traitées.

L'analyse des échantillons de terre et des racines, prélevés après la récolte, dévoila une forte diminution de la population de *Pratylenchus penetrans*.

Les auteurs ont démontré qu'une réinfestation par l'emploi de bulbes, provenant d'un terrain infesté, est possible.

Afin de détruire les nématodes se trouvant à l'intérieur des racines, les bulbes furent soumis à un traitement à l'eau chaude.

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PRATYLENCHUS PENETRANS ALS URSACHE VON „MÜDIGKEITSERSCHEINUNGEN" IN BAUMSCHULEN DER DDR

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Die „Müdigkeitserscheinungen" an landwirtschaftlichen und gärtnerischen Kulturen gehören noch immer zu den umstrittenen Problemen. Man versteht in der Literatur unter „Müdigkeit" überwiegend das Nachlassen der Wachstums- und Ertragsleistung bei wiederholtem Anbau der gleichen Pflanzenart unter Standortsverhältnissen, die eine normale Entwicklung anderer Pflanzenarten ermöglichen. Die Ansichten über die Abgrenzung des Inhaltes der Bezeichnung „Müdigkeit" stimmen allerdings nicht völlig überein. Vielfach werden noch solche Depressionserscheinungen als „Müdigkeit" bezeichnet, die beim Nachbau artverwandter oder auch artfremder Pflanzen auftreten. Sind schon die Meinungen, was man unter „Müdigkeit" zu verstehen hat, nicht einheitlich, so gehen sie hinsichtlich der Ursachen weit auseinander. Drei Theoriengruppen sind in den letzten Jahren in den Vordergrund getreten. Es sind dies die Mangeltheorien, die Toxintheorien und die Organismentheorien (Näheres siehe SCHANDER 1956, DIETER 1957).

Der Wunsch mancher Untersucher, die etwas verworrene Situation endgültig klarzustellen und eine für alle Verhältnisse zutreffende Erklärung zu finden, ist verständlich. Dieser Wunsch darf jedoch nicht zu voreiligen einseitigen Verallgemeinerungen führen. Gerade beim ständigen Anbau mehrjähriger Baumschul- oder Obstkulturen ist durchaus sowohl ein einseitiger Entzug bestimmter Elemente, als auch ein Einfluß der Wurzelzersetzungsprodukte oder der Aufbau einer schädlichen Nematodenpopulation denkbar. Das Erscheinungsbild selbst, das Nachlassen des Wuchses, die Hemmung oder Depression, sagt uns nichts über die Ursachen aus. Deshalb ist es m.E. unzweckmäßig, von der „Müdigkeit" als einer bestimmten Krankheit zu sprechen, d.h. den Kausalitätsbegriff außer Acht zu lassen. Die bisherigen Erkenntnisse zwingen dazu, in jedem Falle des Auftretens von „Müdigkeitserscheinungen", Untersuchungen über die Ursachen anzustellen.

In den nördlichen Bezirken der DDR sind „Müdigkeitserscheinungen" in den Baumschulen keine Seltenheit. Es lag nahe, angeregt durch die Arbeit von OOSTENBRINK (1955) über „Bodenmüdigkeit und Nematoden", einige dieser Objekte in die nematologischen Untersuchungen unseres Institutes einzubeziehen.

Zur Untersuchung gelangten je 1 Baumschule aus den 3 mecklenburgischen Bezirken Rostock, Schwerin und Neubrandenburg. Die Baumschulen sind alle auf leichteren Böden angelegt.

UNTERSUCHUNGEN ÜBER DIE NEMATODENFAUNA „MÜDER" BAUMSCHULQUARTIERE

Für die periodischen Untersuchungen über die Tiefenverteilung der Nematoden wurde ein Birnenquartier mit starken Müdigkeitserscheinungen in Kavelstorf bei Rostock ausgewählt (Abb. 1) [1]). Auf dieser Fläche wurden seit 1912 fast ununterbrochen Baumschulgewächse angezogen. Die ersten Wachstumsdepressionen konnten schon vor über 30 Jahren beobachtet werden. Am stärksten wurden die Apfelbäume geschädigt. Am wenigsten litten die Pflaumenbäume. Die Ackerkrume (lehmiger Sand) ist ca. 30 cm stark. Darunter folgt eine etwa 80 cm starke Lehmschicht auf Sand. Die Bodenreaktion zeigte Werte von pH 6,7 bis pH 6,8. Der Nährstoffgehalt des Bodens ist gut (18 bis 20 mg P_2O_5/100 g Boden, $>$ 50 mg K_2O/100 g Boden).

Die Probenentnahme erfolgte mit einem Bohrstock, der mit einer Zentimetereinteilung versehen war. Dadurch war es möglich, die Proben in 8 Tiefenstufen von je 4 cm zu zerlegen. Jeweils 15 Einstiche wurden zu einer Mischprobe vereinigt. Um Zufallsschwankungen nach Möglichkeit auszugleichen, wurden stets mehrere Mischproben entnommen und deren Ergebnisse später gemittelt. Das Auswaschen der Nematoden erfolgte im Siebtrichterverfahren, einem modifizierten Baermannverfahren.

Die Auswertung der Untersuchungen ergab, daß die Nematodenzahlen in Abhängigkeit von der Bodentiefe und der Jahreszeit großen Schwankungen unter-

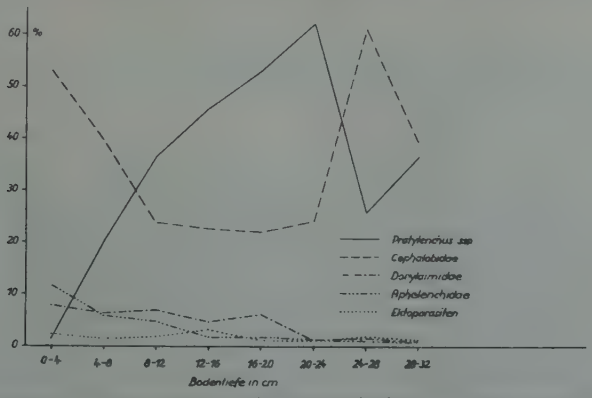

Abb. 2. Die prozentuale Zusammensetzung der Nematodenfauna in verschiedenen Bodentiefen des „müden" Birnenquartiers (Jahresdurchschnitt 1957).

liegen. Die Abb. 2 zeigt die prozentuale Verteilung einiger Nematodengruppen in den verschiedenen Bodentiefen im Jahresdurchschnitt (1957). In der obersten Bodenschicht ist der Anteil der Pratylenchen praktisch gleich Null. Er steigt aber

[1]) Dem Besitzer der Baumschule, Herrn A. H. FLOR, Kavelstorf bei Rostock, sei an dieser Stelle für seine großzügige Unterstützung gedankt.

mit zunehmender Bodentiefe bis auf über 60 % der Gesamtnematodenfauna an, um unterhalb von 24 cm wieder abzufallen. Eine nahezu gegenläufige Tendenz zeigen die Angehörigen der Familie Cephalobidae. Die gleichfalls zusammengefaßten Arten der Familien Dorylaimidae und Aphelenchidae liegen ziemlich auf der gleichen Ebene und spielen wie die Ektoparasiten (*Rotylenchus, Helicotylenchus, Tylenchorhynchus, Paratylenchus, Criconemoides, Xiphinema* und *Trichodorus*) anteilmäßig nur eine unbedeutende Rolle.

Da der prozentuale Anteil nichts über die tatsächlichen Zahlen aussagt, sind in

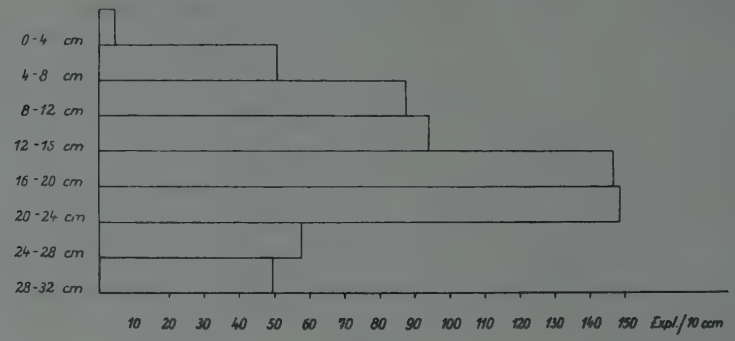

Abb. 3. Die vertikale Verteilung von *Pratylenchus penetrans* im Jahresdurchschnitt.

Abb. 3 die absoluten Werte für die endoparasitischen Pratylenchen graphisch dargestellt.

In der obersten Tiefenstufe von 0 bis 4 cm sind sehr wenig Pratylenchen vorhanden. Es folgt mit der Tiefe eine Zunahme bis zu einem Maximum bei 16-24 cm, in der sich auch die größte Masse der Wurzelsubstanz befindet. Unterhalb dieser Zone gehen die Werte zurück.

Die einzelnen Jahreszeiten zeigen voneinander abweichende Verhältnisse. Die im Jahresdurchschnitt erreichten Werte von ca. 150 *Pratylenchus*-Exemplaren/10 ccm Boden in 16-24 Tiefe werden in der Vegetationszeit noch um 30 bis 50 Exemplare überboten. Im Dezember liegen die absoluten Werte beträchtlich niedriger. Die Reduzierung der *Pratylenchus*-Population zum Winter hin erfolgte in der oberen Bodenschicht von 0-16 cm schneller und stärker als in der unteren von 16-32 cm. In der unteren Schicht sind in allen Jahreszeiten mehr Pratylenchen anzutreffen als in der oberen. Dies ist durch die stärkere Anhäufung der Wurzelsubstanz in der Tiefe unterhalb von 16 cm bedingt.

Die Untersuchungen der engeren Rhizosphäre der Birnenwurzeln im „müden" Quartier ergaben noch höhere Nematodenzahlen. Bis zu 1000 Älchen/10 ccm Boden wurden gezählt. Darunter dominierten die Pratylenchen mit ca. 60 %.

Der *Pratylenchus*-Besatz der Wurzeln (Abb. 4) schwankte zwischen 300 und 800 Exemplaren/1 g Wurzeln. In Teilen des Birnenquartieres mit nur geringen Depressionserscheinungen war der Wurzelbefall beträchtlich geringer (unter 50 Expl./1 g Wurzeln).

Abb. 1. Nematodenherd *(Pratylenchus penetrans)* in einem Birnenquartier, Kavelstorf 1957.

Abb. 4. *Pratylenchus penetrans* in der Wurzelrinde von Apfel (Vergr. 1 : 100).

H. DECKER: *Pratylenchus penetrans in Baumschulen.*

Abb. 5. Borax-Düngungsversuch 1957 (Apfelsämlinge); Links: ungedämpft, *ohne Borax;* Rechts: ungedämpft, *mit* Borax.

Abb. 6. Versuch mit Apfelwurzelzugaben 1957 (Typ EM XI, 1-jährig); Links: gedämpft, *ohne* Wurzelzusatz; Rechts: gedämpft, *mit* Wurzelzusatz.

Abb. 7. Nematodeninfektionsversuch 1957 (Typ EM XI, 1-jährig); Links: gedämpft, *ohne* Nematodenzugabe; Rechts: gedämpft, *mit* Nematodenzugabe.

Artbestimmungen ergaben, daß *Pratylenchus penetrans* (Cobb, 1917) Sher and Allen, 1953 vorherrschte. Nur vereinzelt konnte *Pr. pratensis* (de Man, 1880) Filipjev, 1936 festgestellt werden. Das zahlenmäßige Verhältnis von Männchen zu Weibchen betrug bei *Pr. penetrans* im Durchschnitt 1 : 5. Im Freiland war das Verhältnis weiter (1 : 8,4) in den Gefäßen enger (1 : 3,5). Auch in den Wurzeln war das Verhältnis von Männchen zu Weibchen mit gewissen Abweichungen 1 : 5.

Die gefundenen Werte dürfen nun nicht als absolut feststehend, unveränderlich angesehen werden. Wie alle anderen Dinge in der Natur so unterliegen auch die Nematodenpopulationen der ständigen Einwirkung der Umwelt. Je nach Stärke der auf sie einwirkenden Umweltfaktoren wird eine Beeinflussung erfolgen. Es steht außer Zweifel, daß den Nahrungs- und Klimafaktoren dabei die größte Bedeutung zukommt. Die Konzentration der Parasiten in bestimmten von der Wurzelsubstanz abhängigen Bodentiefen und die Reduzierung der Nematodenpopulation in den Wintermonaten sind Ausdruck dieser Abhängigkeiten.

GEFÄSSVERSUCHE ZUR FRAGE DER URSACHE VON „MÜDIGKEITSERSCHEINUNGEN" IN BAUMSCHULEN

Die Höhe der *Pratylenchus*-Population im Boden und in den Wurzeln und die Konzentration dieses Parasiten im Wurzelbereich lassen eine kausale Beziehung zu den „Müdigkeitserscheinungen" vermuten. Ein Beweis kann jedoch nur durch experimentelle Untersuchungen erbracht werden. Aus diesem Grunde wurden in den Jahren 1956 und 1957 Gefäßversuche mit Boden aus mehreren „müden" Baumschulen durchgeführt.

Als Versuchspflanzen dienten Apfelsämlinge, *Malus*-Unterlagen Typ EM XI (1- und 2-jährig) und 1-jährige *Malus*-Unterlagen Typ EM IX. Die Pflanzung erfolgte jeweils Ende April/Anfang Mai in eingegrabenen Tontöpfen (22 cm ø). Ein Teil des Bodens war 3 Wochen zuvor gedämpft worden (50 Minuten bei 95-99° C).

Der Einfluß der Dämpfung machte sich bereits während der Vegetationszeit durch starke Wachstumsunterschiede bemerkbar. Der Längenzuwachs der „müden" Pflanzen betrug z.B. 1956 bei Typ EM XI im Vergleich zu den Pflanzen im gedämpften Boden nur 60,7 %, im Jahre 1957, also im 2. Jahr, 51 %. Im gedämpften Boden blieb der Zuwachs in beiden Jahren gleich. Die Dämpfung bewirkte bei allen geprüften Unterlagen und bei den Sämlingen einen statistisch sehr gut gesicherten Unterschied im Längenwachstum.

Die jeweils am Ende der Vegetationszeiten vorgenommenen Analysen der Nematodenfauna in den Versuchsgefäßen ließen keinen Unterschied in der Gesamtnematodenzahl zwischen gedämpftem und ungedämpftem Boden erkennen. Nur der Anteil der Pratylenchen weicht voneinander ab. Während *Pratylenchus penetrans* in den ungedämpften Reihen stärker vertreten ist, kommt er in den gedämpften kaum vor.

Auch in den Wurzeln zeigte sich das gleiche Bild.

Nematodenbesatz pro 1 g Wurzeln

	ungedämpft (n = 53)	gedämpft (n = 54)
Gesamtnematoden	50,2 Expl.	20,2 Expl.
davon *Pratylenchus penetrans*	32,7 Expl.	0,6 Expl.

In den Wurzeln beider Reihen kamen außerdem noch *Aphelenchus avenae* Bastian, 1865, *Aphelenchoides ssp., Tylenchus ssp.* (besonders *T. thornei* Andrássy, 1954) und Angehörige der Cephalobidae in wechselnder Anzahl vor.

In den angestellten Versuchen wurde auch die Frage geprüft, ob eine Mineraldüngung oder eine Boraxgabe einen Einfluß auf das Wachstum der Versuchspflanzen ausübt. Bei der Auswertung der Versuche ergab sich, daß im ungedämpften Boden weder durch eine mineralische Düngung noch durch eine Boraxgabe (60 mg, 40 mg, 30 mg/Versuchsgefäß) das Längenwachstum und der *Pratylenchus*-Besatz beeinflußt wird (Abb. 5).

Nach Ansicht der Vertreter der Toxintheorie sind es bestimmte Wurzelzersetzungsprodukte, die als Ursache der „Müdigkeitserscheinungen" zu gelten haben. Verschiedene Autoren (Vogel 1939, Fastabend 1954) berichteten über die künstliche Erzeugung von „Müdigkeitserscheinungen" durch Zugabe von Wurzeln. Diesen positiven Angaben stehen aber auch negative Mitteilungen gegenüber (Swart-Füchtbauer 1954).

In den eigenen Versuchen wurden die Wurzeln von 2-jährigen gesunden Apfelunterlagen fein zerschnitten in die nächste Umgebung des Wurzelwerks der in gedämpftem Boden gepflanzten Versuchsgewächse gebracht. Für jedes im Versuch stehende Gefäß wurde die gesamte Wurzelsubstanz eines frisch ausgegrabenen Bäumchens verwendet. Um die spezielle Pilz- und Bakterienflora zu erhalten, wurden die eingebrachten Wurzeln vorher nicht gereinigt. Es erfolgte aber eine stichprobenartige Untersuchung der Wurzeln auf *Pratylenchus*-Befall. Dies ist notwendig, um zu verhindern, daß mit den Wurzeln adulte Tiere, Larven oder Eier von parasitischen Nematoden in die Versuchsgefäße eingeschleppt werden und eine Wurzelwirkung vorspiegeln. Die für unsere Versuche benutzten Wurzeln waren befallsfrei.

Die Auswertungen ließen im großen und ganzen keine Beeinflussung auf den Pflanzenwuchs erkennen (Abb. 6). In einigen Versuchsreihen zeigte sich ein etwas verringertes Längenwachstum, in einigen anderen Reihen dagegen durch den Wurzelzusatz eine geringe Wachstumsverbesserung. Da eine statistische Sicherung in keinem Falle möglich war, müssen die Abweichungen als Zufallsschwankungen angesehen werden. Eine Beeinflussung des Nematodenbesatzes durch die Wurzelzugabe erfolgte nicht. Der *Pratylenchus*-Gehalt der Wurzeln im gedämpften Boden überstieg niemals 1 Expl./1 g Wurzeln.

In weiteren Versuchsreihen wurden Infektionen mit *Pratylenchus penetrans*

durchgeführt. Jedes im Versuch stehende Gefäß wurde mit einer Nematodensuspension, die etwa 2500 Pratylenchen enthielt, beimpft. Die pathogenen Pratylenchen sind als die biologisch stärker spezialisierten Arten gegen Änderungen ihrer Lebensbedingungen empfindlicher als die Saprozoiten, so daß bei Infektionsversuchen mit Mißerfolgen gerechnet werden muß. Hierauf haben auch schon OOSTENBRINK, s'JACOB & KUIPER (1957) aufmerksam gemacht. Auch in unseren Versuchen gelangen nicht alle Infektionen.

Von den gelungenen Infektionen sollen nachstehend die Ergebnisse mit Typ EM XI (einjährig) in gedämpftem Kavelstorfer Boden wiedergegeben werden: (Abb. 7).

1957	ohne Nematoden (n = 12)	mit Nematoden (n = 12)				
	$\bar{x}$	$\bar{x}$	$\bar{x}_D$	FG	t	p*)
Längenzuwachs	23,7 cm	9,3 cm	14,4 cm	11	3,8	0,30 %
Pratylenchus-Besatz/10 ccm Boden	0,6 (n = 12)	6,25 Exemplare (n = 12)				
Pratylenchus-Besatz/1 g Wurzeln	0,25 (8 g)	37,0 Exemplare (6 g)				

*) Die Verrechnung erfolgte nach der Differenzmethode (KUCKUCK-MUDRA 1950).
$\bar{x}$ = Mittelwert, $\bar{x}_D$ = Mittelwert der Einzeldifferenzen.

FG = Freiheitsgrad (= n — 1) $t = \frac{D \text{ (= Einzeldifferenzen)}}{m_D \text{ (= Fehler der Differenz)}}$

p = Grenzwahrscheinlichkeit, p = > 5 % nicht gesichert; p = 5—1 % schwach gesichert, p = 1—0,2 % gesichert, p = 0,2 >—0,10 % gut gesichert, p = < 0,10 % sehr gut gesichert.

Die in den beimpften Gefäßen aufgetretenen Wachstumshemmungen unterschieden sich nicht von den „Müdigkeitserscheiungen" im ungedämpften Boden. Auch bei den Sämlingen sowie z.T. bei den 2-jährigen Unterlagen Typ EM XI konnten statistisch gesicherte Infektionserfolge erzielt werden.

In den mißglückten Infektionsversuchen hatten sich die Pratylenchen nicht vermehrt, sondern waren fast völlig verschwunden. Die Wurzeln selbst waren befallsfrei. Es ließen sich auch keine Wachstumsunterschiede zu den Kontrollpflanzen feststellen. Diese „mißglückten" Infektionsversuche unterstreichen ebenfalls die kausale Rolle der Pratylenchen bei der Entstehung von Wachstumshemmungen.

DISKUSSION

Die wachstumssteigernde Wirkung einer Dämpfung ist schon lange bekannt. Der bessere Wuchs wird einerseits durch die Aufschließung bestimmter pflanzennotwendiger Substanzen, andererseits durch die Beseitigung wachstumshemmender

thermolabiler Stoffwechselprodukte und durch die Vernichtung schädlicher Organismen bedingt. Für die vorliegenden Versuche möchte ich besonders den letzten Punkt herausstellen. Wie die Boden- und Wurzeluntersuchungen ergaben, werden durch die Dämpfung besonders die phytopathogenen Pratylenchen betroffen. Die Wiederbesiedlung der gedämpften Erde mit saproben Nematoden erfolgt relativ schnell.

Die als typisches Kennzeichen einer stärkeren „Müdigkeitserscheinung" angegebene Wirkungslosigkeit einer mineralischen Düngung auf den Längenzuwachs war auch in den eigenen Versuchen zu beobachten.

Von KOBERNUSS (1952) wurde Bormangel als Ursache der Obstbaummüdigkeit angegeben. Die eigenen Borax-Düngungsversuche verliefen ergebnislos. Bormangel kann demnach als Ursache der „Müdigkeitserscheinungen" in den von uns untersuchten Baumschulen nicht in Frage kommen.

Unsere Versuche, durch Zugabe von zerkleinerten Apfelwurzeln zu gedämpftem Boden eine „Müdigkeit" zu erzeugen, schlugen fehl. Damit soll keineswegs die Möglichkeit der Entstehung von Entwicklungshemmungen durch Wurzelzersetzungsprodukte bestritten werden. Sehr wahrscheinlich spielt der Boden für das Wirksamwerden von hemmenden Wurzelzersetzungsprodukten die entscheidende Rolle.

Die Ergebnisse unserer Infektionsversuche mit *Pratylenchus penetrans* sind eindeutig. In den Gefäßen, in denen die Pratylenchen zur Vermehrung gelangten und die Wurzeln befallen hatten, kümmerten auch die Pflanzen. Die Wurzeln waren stark nekrotisiert. In den „mißlungenen" Infektionsversuchen, in denen keine Vermehrung der Pratylenchen und auch kein Wurzelbefall festgestellt werden konnte, trat auch keine Hemmung im Pflanzenwuchs auf. Die Wurzeln besaßen eine gesunde helle Farbe.

Die Ergebnisse gestatten es, die aufgetretenen Wachstumshemmungen mit dem *Pratylenchus*-Befall der Wurzeln in eine kausale Beziehung zu setzen. Sie erlauben auch die entsprechenden Schlußfolgerungen für die untersuchten „müden" Quartiere mit dem starken *Pratylenchus*-Befall zu ziehen. Als Ursache der „Müdigkeit" muß Wurzelbefall durch *Pratylenchus penetrans* angesehen werden.

Es erscheint angebracht, nach der Feststellung der Ursache nicht mehr von „Müdigkeit", sondern von Nematodenschäden zu sprechen.

SUMMARY

Pratylenchus penetrans as the cause of soil-sickness in tree nurseries of the DDR

Soil-sickness is not rare in tree nurseries in the northern districts of DDR. Repeated investigations of the nematode fauna in sick soil show its relation to soil depth and season of the year. Most *P. penetrans* were found at a depth of 16-24 cm, i.e. the zone containing most roots. The highest values are found during the summer, the lowest during the winter. Present theories of the causes of soil sickness were tested by means of extensive pot experiments with various apple stocks. Plant-growth in sick soil could not be improved by adding borax to the soil. Adding nematode-free apple-roots to steam-treated soil sometimes caused weak but statistically insignificant stunting. Artificial infestation of steam-treated soil caused stunting of the growth as much as in sick soil. No stunting was observed if the parasites died after infestation was carried out and the roots had not been attacked.

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POPULATIONSVERÄNDERUNGEN DES KARTOFFELNEMATODEN (*HETERODERA ROSTOCHIENSIS* WOLL.) BEIM ANBAU NEMATODENRESISTENTER UND NEMATODENANFÄLLIGER KARTOFFELSORTEN UNTER BERÜCKSICHTIGUNG DES AUFTRETENS AGGRESSIVER BIOTYPEN [1])

VON

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Durch die Beobachtung ELLENBYS (1952), daß unter den von ihm geprüften knollenbildenden Wildarten der Gattung *Solanum* einige Herkünfte von *Solanum andigena* und *S. ballsii* (jetzt als *S. andigena* subsp. *ballsii* bezeichnet) eine beachtliche Resistenz gegenüber *Heterodera rostochiensis* aufzuweisen haben, gewann das Problem der Züchtung nematodenresistenter Kartoffelsorten erneut an Interesse. Von besonderer Bedeutung war dabei die Feststellung, daß die Wurzelsekrete mancher resistenter Herkünfte sogar die Larven von *H. rostochiensis* zum Schlüpfen anregten. Die Larven drangen auch in die Wurzeln ein, kamen aber hier im allgemeinen nicht zur Zystenbildung. Da den resistenten Pflanzen somit eine Feindpflanzenwirkung zukommt, muß sie sich zweifellos auch auf die Populationsdynamik des Kartoffelnematoden auswirken. Schon HUYSMAN (1957) stellte eine Verminderung des Larvenbesatzes je Zyste von durchschnittlich 70 % bereits nach einmaligem Anbau eines widerstandsfähigen Kartoffelstammes fest; nach zweimaligem Anbau betrug die Abnahme sogar 85 %. WILLIAMS (1958) erreichte durch den Anbau des resistenten Stammes Z B/7 im ersten Jahre einen Befallsrückgang je Bodeneinheit von 48 %, im zweiten Jahre eine Senkung um 83 % der Anfangsverseuchung. STELTER & RAEUBER (1959) erzielten nach einer vor kurzem erschienenen Veröffentlichung bei resistenten Varietäten in einem Jahre einen Befallsrückgang von 84-88 %.

Diese Ergebnisse wurden anscheinend sämtlich mit dem Normaltyp des Kartoffelnematoden erzielt. Nachdem dann zuerst DUNNETT (1957) in Schottland, dann JONES (1958) in England Biotypen des Kartoffelnematoden nachweisen konnten und ihr Vorkommen auch in Holland (HUYSMAN, 1958) und Deutschland (SCHICK, siehe HENNIGER (1958), GOFFART (1958)) beobachtet wurde, erhob sich die Frage, ob und in welchem Umfange die Nematodenpopulationen

[1]) Der Deutschen Forschungsgemeinschaft sei für die Bereitstellung von Mitteln auch an dieser Stelle gedankt.

auch beim Vorkommen solcher Biotypen durch den Anbau resistenter Kartoffelsorten verändert werden.

In der vorliegenden Mitteilung soll über Versuche berichtet werden, die wir in den Jahren 1958 und 1959 zur Frage der Populationsverschiebungen beim Anbau resistenter Kartoffelsorten durchgeführt haben und bei denen neben dem Normaltyp des Kartoffelnematoden in einigen Fällen auch resistenzbrechende Biotypen vorhanden waren.

METHODIK

Aus zeitbedingten Gründen wurde in beiden Jahren nur mit dem resistenten Stamm 18/53 gearbeitet. Dieser ist eine Kreuzung von *Solanum tuberosum* var. Oberarnbacher Frühe und einem Bastard aus *S. tuberosum* var. Record x *S. andigena* CPC 1673-1. In Vorversuchen, die auf das Jahr 1956 zurückgehen, hat dieser Zuchtstamm nicht nur eine beachtliche Resistenz gegenüber Kartoffelnematoden gezeigt, sondern sich als eine Neuzüchtung erwiesen, die auch in anderer Hinsicht, z.B. hinsichtlich ihres Ertrages und ihrer Verwendungsmöglichkeiten, bemerkenswerte Eigenschaften besitzt. Im Frühjahr 1958 standen uns 200 kg Saatgut als Hochzucht zur Verfügung. Für die Durchführung der Versuche wurden 8 gut verseuchte Felder ausgewählt, von denen 4 im Land Niedersachsen, 4 im Land Nordrhein-Westfalen weit voneinander verstreut lagen. Von keinem Feld war vorher bekannt, ob es einen Biotyp des Kartoffelnematoden enthält oder nicht. Vor Anlage der Versuche wurden Bodenproben gezogen und die Verseuchung an Eiern und Larven in je 100 g Boden ermittelt. Dann folgte die Bestellung der Flächen teils mit der resistenten Neuzüchtung 18/53, teils mit einer in der jeweiligen Anbaulage häufiger benutzten frühen oder mittelfrühen, aber anfälligen Handelssorte. Zur Zeit der Zystenreife wurden die Wurzeln von wenigstens 50 resistenten Stauden zunächst visuell am Ort auf Zystenbehang bonitiert und dann bis zu 10 Pflanzen mit anhaftender Erde entnommen, um sie im Labor noch sorgfältiger zu untersuchen. Hierbei wurden von den resistenten Sorten je Staude 1 kg Erde auf frische und alte Zysten geprüft. Bei den anfälligen Vergleichssorten beschränkten wir uns auf die Untersuchung von 3 x 100 g Boden je Staude.

ERGEBNISSE

Aus Tabelle I ergibt sich, daß nach Anbau von 18/53 in den meisten Fällen keine Neubildung von Zysten erfolgte. Der Inhalt der alten Zysten lag durchweg niedriger als nach Anbau einer anfälligen Sorte. Das deutet auf ein sehr starkes Aktivierungsvermögen des Zuchtstammes hin. Die Zahl der Zysten ist zwar weniger stark zurückgegangen, was bei der längeren Lebensdauer nicht ungewöhnlich ist; im Gesamtergebnis war aber der Anteil an infektiösen Larven im Herbst um mehr als 96 % der Anfangsverseuchung im Frühjahr zurückgegangen. Bei der bekannten auf kleinstem Raum vorhandenen Ungleichmäßigkeit der Zystenverteilung im Boden ist der hohe Rückgang in der Populationsdichte, der bei allen Versuchen mehr als 90 % betrug, erstaunlich.

TABELLE I

Populationsveränderungen des Kartoffelnematoden beim Anbau resistenter und anfälliger Sorten 1958

	Zystenzahl in 100 g Boden v. d. Bestellung	Sorte	Neugebildete Zysten in 100 g Rhizosph. erde	Alte Zysten in 100 g Rhizosph. erde	Insgesamt	Eier und Larven in % des Ausgangswertes	% „Brecher"
Br.	59 (4700)	18/53	0 (0)	58 (109)	58 (109)	2,4	0
		B.	135 (31350)	55 (124)	190 (31474)	570	
Sa.	44 (2200)	18/53	0 (0)	69 (192)	69 (192)	8,7	0
		B.	201 (32850)	69 (188)	270 (33038)	1502	
Me.	101 (10300)	18/53	1 (217)	54 (237)	55 (454)	4,4	1
		B.	78 (20518)	57 (227)	135 (20745)	201	
Er.	107 (8450)	18/53	0 (0)	62 (265)	62 (265)	3,1	0
		Ob.	435 (100225)	87 (288)	522 (100513)	1189	
Hi.	139 (13200)	18/53	2 (332)	138 (585)	140 (917)	6,9	1,5
		Gr.	380 (95260)	178 (964)	558 (96224)	729	
Mü.	82 (6150)	18/53	0 (0)	41 (13)	41 (13)	0,2	0
		Gr.	89 (23680)	26 (23)	115 (23703)	385	
Pe.	45 (4090)	18/53	0 (0)	32 (5)	32 (5)	0,1	0
		B.	288 (7275)	34 (159)	322 (7434)	182	
Schi.	33 (5665)	18/53	0 (0)	30 (5)	30 (5)	0,1	0
		E.	12 (2950)	44 (6)	56 (2956)	52	
M	76 (6844)		< 1 (69)	61 (176)	61 (245)	3,2	
			202 (39263)	69 (247)	271 (39510)	614	

Erklärung: B. = Bona, E. = Erstling, Gr. = Grata, Ob. = Oberarnbacher Frühe. In ( ) Zahl der Eier.

Unter den aufgeführten Herkünften enthielten die Versuchsstellen Me und Hi einen kleinen Anteil „Resistenzbrecher", der jedoch 1,5 % nicht überschritt. Auch in diesen beiden Fällen lag die Endverseuchung mit 4,4 bzw. 6,9 % der Anfangsverseuchung nur wenig über dem Mittelwert (3,2 %). Dieses günstige Ergebnis kann möglicherweise den Witterungsbedingungen zuzuschreiben sein, die für das Schlüpfen der Larven im Jahre 1958 besonders günstig waren. Wir wiederholten daher die Versuche im Jahre 1959 an anderen stark infizierten Stellen. Außer in Niedersachsen und Nordrhein-Westfalen wurden Versuche auch im hessischen Raum durchgeführt. Anlage und Auswertung erfolgten wie im Vorjahre. Für 5 Versuche liegt das vollständige Zahlenmaterial vor (Tab. II). In 4 Versuchen konnten wir eine Zystenneubildung beobachten, die jedoch — abgesehen von einem Fall — so niedrig war, daß sie sich in der Populationsdynamik nicht nennenswert

Abb. 1. Einfluss des Anbaues von 18/53 auf den Nachbau einer anfälligen Kartoffelsorte. Vorn und hinten: Sorte Bona nach 18/53, mitte Bona nach Grata.

auswirken konnte, da die Zahl der „Brecher" zwischen 1 und 2 % lag. Der Rückgang betrug auch hier mehr als 90 %. Besondere Beachtung muß jedoch dem Versuch Ha geschenkt werden, weil wir hier in 100 g Boden 20 frische Zysten mit einem Inhalt von 3430 Eiern fanden und 7 % „Brecher" ermitteln konnten. Der Befall war schon rein äußerlich am Wurzelballen deutlich erkennbar. Bei der Feldbonitur hatte man sogar den Eindruck, als ob zwischen der resistenten

TABELLE II

Populationsveränderungen des Kartoffelnematoden beim Anbau resistenter und anfälliger Sorten 1959

	Zystenzahl in 100 g Boden v. d. Bestellung	Sorte	Neugebildete Zysten in 100 g Rhizosph. erde	Alte Zysten in 100 g Rhizosph. erde	Insgesamt	Eier und Larven in % des Ausgangswertes	% „Brecher"
Altj.	84 (407)	18/53	0,3 (63)	93 (0)	93 (63)	7,8	1
		Petra	32 (4446)	122 (329)	154 (4775)	595	
Fl.	111 (12383)	18/53	2 (437)	75 (473)	77 (910)	7,3	2
		Bona	94 (18500)	56 (652)	150 (19152)	155	
Schi. (2)	21 (2792)	18/53	0,3 (68)	18 (90)	18 (158)	5,7	< 1,5
		Bona	52 (7980)	38 (211)	90 (8191)	293	
Ha.	200 (10000) *)	18/53	20 (3430)	221 (46)	241 (3476)	34,8	10
		Bona	292 (33113)	198 (136)	490 (33249)	332	
F.Ho.	70 (3500) *)	18/53	0 (0)	30 (173)	30 (173)	4,9	0
		Fina	142 (28870)	70 (403)	212 (29273)	836	

*) geschätzt

und der anfälligen Sorte (Bona) kaum ein wesentlicher Befallsunterschied vorlag, doch ergab die genaue Auszählung von 3 x 100 g Boden bei der Vergleichssorte Bona doch noch eine fast 10 mal so hohe Bodenverseuchung. Der Rückgang auf 34,8 % der Frühjahrsverseuchung ist selbstverständlich als unzureichend anzusehen.

Als Ursache stärkeren Befalls wird in der Regel die mutative Entstehung aggressiver Biotypen angenommen. Diese Annahme dürfte auch für den letztgenannten Fall zutreffen. Ob der schwächere Befall an resistenten Pflanzen, wie wir ihn 1958 und 1959 in mehreren Fällen beobachten konnten, ebenfalls so erklärt werden muß, läßt sich noch nicht sagen. Es bestehen jedoch gewisse Anzeichen, ihn auf rein phänotypische Ursachen zurückzuführen und ihn in Abhängigkeit zu verschiedenen ökologischen Faktoren zu bringen. So fällt in den Versuchen auf, daß ein Aggressionsvermögen mit einer sehr hohen Anfangsverseuchung parallel geht. Es wäre auch denkbar, daß ein starker *Rhizoctonia*-Befall, wie er im Versuch Fl in Erscheinung trat, eine verminderte Resistenz der Pflanzen zur Folge hat. Schließlich können Veränderungen bei Parasit und Wirtspflanze auch zusammentreffen.

Für die Annahme phänotypischer Einflüsse sprechen weiterhin folgende Feststellungen:

1. Zysten, die nach ihrem Aussehen als vollvital angesehen werden mußten und von resistenten Stämmen selektiert worden waren, kamen an resistenten Stämmen nicht immer zur Vermehrung, während sie an einer anfälligen Vergleichssorte sogleich Zysten bildeten.

2. In wiederholten Versuchen mit aggressiven Stämmen trat bei gleicher Versuchsmethodik in einzelnen Fällen Befall ein, in anderen Fällen jedoch nicht, ohne daß dafür eine Erklärung gegeben werden kann.

Über die Verteilung der „Resistenzbrecher" läßt sich noch wenig aussagen. Den Herd mit dem stärksten Anteil an „Brechern" fanden wir im Vorgebirge der Rhön, und es ist vielleicht ganz interessant zu wissen, daß die bisherigen Funde vorwiegend aus dem Thüringer Wald bekanntgeworden sind (SCHICK, siehe HENNIGER 1958). Aus dem gleichen Gebiet wurden übrigens auch aggressive Biotypen des Kartoffelkrebses (*Synchytrium endobioticum*) bekannt. Das soll aber nicht ausschließen, daß Biotypen des Kartoffelnematoden auch in der Ebene vorkommen können.

Der Anbau des Zuchtstammes 18/53 wirkte sich auch beim Nachbau einer anfälligen Handelssorte noch günstig aus. Abb. 1 zeigt vorn und hinten den Einfluß von 18/53 auf den Nachbau (Bona), in der Mitte den Einfluß zweimaligen Anbaues einer anfälligen Sorte (1958 Grata, 1959 Bona). Der Unterschied macht sich auch in der Populationsdichte geltend, wie eine Untersuchung bei der Ernte im August 1959 ergab (Tab. III).

Tabelle III

Wirkung eines einmaligen Anbaues von 18/53 mit nachfolgender anfälliger Kartoffelsorte auf die Populationsdichte (August 1959)

Links 1958: 18/53 1959: Bona		Mitte 1958: Grata 1959: Bona		Rechts 1958: 18/53 1959: Bona	
Zysten je 100 g Boden		Zysten je 100 g Boden		Zysten je 100 g Boden	
frisch	alt	frisch	alt	frisch	alt
77	243	137	212	39	88
138	255	203	245	138	132

In einem weiteren Versuch konnten wir den Einfluß eines zweimaligen Anbaues des Zuchtstammes 18/53 auf die Nematodenpopulation verfolgen (Tab. IV).

Tabelle IV

Populationsveränderung nach zweimaligem Anbau des Zuchtstammes 18/53

	Eier und Larven je 100 g Boden		
	Frühjahr 1958	Herbst 1958	Herbst 1959
18/53	13200	917	132
Grata	13200	96224	52564

Bei einer Populationsdichte von 13200 Eiern je 100 g Boden im Frühjahr 1958 beobachteten wir nach Anbau von 18/53 im Herbst 1958 einen Rückgang auf 917 Eiern (= 93 %); nach einem nochmaligen Anbau wurde ein weiterer Rückgang von 86 % gegenüber Herbst 1958 erzielt, sodaß am Ende des zweiten Anbaujahres die effektive Bodenverseuchung mit 132 Eiern je 100 g Boden nur noch 1 % der Populationsdichte vom Frühjahr 1958 betrug.

Der Anbau von 18/53 übt nach den bisherigen Beobachtungen in den meisten Fällen auch einen stärkeren Schlüpfreiz auf die im Boden befindlichen Larven aus als eine der angebauten Vergleichssorten. Das geht aus den Berechnungen des Inhalts der alten Zysten hervor. Wir haben in Tab. V die Mittelwerte aus

Tabelle V

Reizwirkung des Zuchtstammes 18/53 auf den Inhalt älterer Zysten

	Eier und Larven in alten Zysten je 100 g Boden		
		Vergleichswerte	Diff.
M aus 8 Versuchen 1958	176	247	— 29%
M „ 5 „ 1959	156	346	— 55%

den Versuchen der Jahre 1958 und 1959 (Spalte) errechnet und sie den Mittelwerten aus den Vergleichssorten gegenübergestellt. Sie betrugen 1958 29 %, 1959 55 %. Fassen wir die Ergebnisse unserer zweijährigen Beobachtungen zusammen, so ergibt sich folgendes:

1. Der Anbau des nematodenresistenten Zuchtstammes 18/53 hat die Populationsdichte in den meisten Fallen um mehr als 90 % der Anfangsverseuchung gesenkt.

2. „Resistenzbrecher" traten 1958 und 1959 auf. Sie erreichten 1958 höchstens 1,5 %. In den Versuchen des Jahres 1959 wurden 4 Herde mit „Brechern" gefunden. Der stärkste im Vorgebirge der Rhön gelegene Herd hatte 10 % „Brecher". In diesem einen Falle hat der Anbau des Stammes 18/53 die Bodenverseuchung nur um 65 % vermindert. Bei den übrigen Herkünften betrug der Prozentsatz an „Brechern" 1 bis 2 %, und der Befallsrückgang lag über 90 %.
3. Das Auftreten resistenzbrechender Formen kann genotypisch auf dem Wege der Mutation erfolgen. Es scheinen aber auch phänotypische Ursachen hierfür in Betracht kommen zu können (Steigerung des Infektionsdrucks des Parasiten oder durch äußere Einflüsse bedingte verminderte Resistenz der Pflanzen).
4. Bei einem unmittelbaren Nachbau des Stammes 18/53 ging die Bodenverseuchung im Herbst des zweiten Jahres beim Normaltyp des Kartoffelnematoden bis auf 1 % der Ausgangsverseuchung zurück.
5. Die Wurzelsekrete des Zuchtstammes 18/53 wirken stärker schlüpffördernd als die mehrerer anfälliger Handelssorten. Infolgedessen kommt es auch schneller zur Entleerung der Zysten.

SUMMARY

The effect of the cultivation of nematode-resistent and nematode-susceptible potato varieties on the population of potato-root nematode (Heterodera rostochiensis *Woll.*) *with special reference to the appearance of aggressive biotypes*

Cultivation of the hybrid 18/53 [*Solanum tuberosum* var. Oberarnbacher Frühe x (*S. tuberosum* var. Record x *S. andigena* CPC 1673-1)] gave a reduction of 90 % in the population level of the potato cyst nematode in the first year. "Resistance-breakers", which constitute only 1,5 % of the population, did not affect the decrease; with a higher proportion of "resistance-breakers" the decline might be smaller. The occurrence of "resistance-breakers" appear to be both genotypic and phenotypic. The root exudate from var. 18/53 gave a greater hatch than of existing potato varieties. After cultivating 18/53 for two years the level of infection had fallen in one case by 99 %.

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POTATO BREEDERS' STRAINS OF ROOT EELWORM (*HETERODERA ROSTOCHIENSIS* WOLL.)

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The first phase of breeding for resistance to potato root eelworm was initiated by the finding of resistance in clones C.P.C. 1673, C.P.C. 1685 and C.P.C. 1690 of *Solanum tuberosum* L. subsp. *andigena* Juz. et Buk. (ELLENBY, 1952). However, none possessed uniform resistance to populations from different fields, glasshouses and gardens in Britain (DUNNETT, 1957; JONES, 1957). DUNNETT (1958) selected clones of *S. vernei* Bitt. et Wittm., *S. multidissectum* Hawkes and *S. sanctae-rosae* Hawkes, three diploid species of potato, as potentially useful sources of resistance to the so-called Duddingston population of potato root eelworm maintained at the Scottish Plant Breeding Station and characterised by aggressiveness towards plants possessing the resistance of subsp. *andigena* C.P.C. 1673. The latter type of aggressiveness distinguished the Duddingston population from the Boghall population, also maintained at the Scottish Plant Breeding Station. Further study showed that the Boghall population was aggressive towards plants possessing the resistance which was present in the selected clones of *S. multidissectum*. The selected clones of *S. vernei* and *S. sanctae-rosae* were equally resistant to both populations. These findings have prompted an enquiry into the status of physiological strains of the eelworm and the possibility of investigating the relationship between genes controlling pathogenicity in the eelworm and genes controlling resistance in potatoes.

THE SELECTIVE CULTURE OF BREEDERS' STRAINS OF THE POTATO ROOT EELWORM

A selective host for an aggressive population of the eelworm may be defined as a clone or variety of potato possessing resistance which discriminates against the survival of biotypes lacking the type of aggressiveness which is the distinguishing feature of the population. Consequently, plants possessing the resistance of subsp. *andigena* C.P.C. 1673 can be regarded as selective hosts for the Duddingston population, which has been multiplied annually for the past four years on plants having this type of resistance. Therefore the present Duddingston population can be denoted by Duddingston x 4 (C.P.C. 1673) and *population x n* (*selective host*) denotes the general case.

The efficiency of selective culture is greatest if cysts containing an unhatched

residue of viable eggs of the parental generation do not mingle with those of their progeny. Separation can be achieved by using prehatched larvae as inoculum or by enclosing the parental cysts in a wire basket of appropriate mesh (ELLENBY, 1955).

If recessive aggressiveness is postulated, a population composed solely of biotypes homozygous for aggressiveness is achieved when $n = 1$, provided (a) that one of the foregoing techniques for selective culture is employed and (b) that the survival of males and females is dependent on the same genetic factors, in which case only recessive males would survive to fertilise the recessive females. If dominant aggressiveness is postulated and an equal frequency of the gene *A* for aggressiveness and the gene *a* for non-aggressiveness is assumed initially, then random mating in a selectively cultured population of unlimited size results in the following trend towards homozygous aggressiveness: —

$$\text{population} \times n \text{ (selective host)} = (n + 1)^2\ AA + 2\ (n + 1)\ Aa + 1aa.$$

This is an adaptation of the formula given by DICE (1940) in a paper on the elimination of lethal characters in populations. By analogy with Dice's exposition the trend towards homozygosity would take place at approximately the same rate whether aggressiveness were controlled either by multiple dominant factors or by a single dominant gene, as instanced above. If some of the multiple factors were recessive, the trend towards homozygosity would be more pronounced.

Granted that a particular population of the eelworm is composed almost entirely of biotypes which are aggressive towards a particular selective host, a true measure of aggressiveness is the probability of a given biotype surviving to female maturity in the selective host. Prolonged selective culture will not increase the rate of multiplication of a population which is already homozygous for a low degree of aggressiveness unless mutation in the direction of increased aggressiveness takes place.

An aggressive population which is subjected to selective culture may be regarded justifiably as a breeders' strain of the eelworm but the classification of different strains, each having a different selective host, is complicated by the fact that resistance or aggressiveness must be measured against a continuous scale. It is proposed to restrict the differential host series to plants possessing at least a certain "standard" level of resistance to one or more strains, to ensure that the classification is the basis of a practical scheme for controlling potato root eelworm by varietal resistance in potatoes.

A STANDARD LEVEL OF RESISTANCE TO EELWORM

The clone C.P.C. 1673 of subsp. *andigena* incorporated a dominant gene designated *H* by TOXOPEUS & HUIJSMAN (1953), which conferred resistance to eelworm. Even before the finding of aggressive populations of the eelworm in Britain, there was abundant evidence that cysts arose occasionally on plants of genotype *H*, (TOXOPEUS & HUIJSMAN, 1953; JONES, 1954; WILLIAMS, 1956; COLE & HOWARD, 1957): in particular, "occasional" cysts were observed on the resistant plants

exposed to infection by the Boghall population of the eelworm (DUNNETT, 1957). The latter relationship was further studied to establish a practical basis for the measurement of a "standard" level of resistance to eelworm, corresponding to the level of resistance which was available to potato breeders during the first phase of breeding for resistance to eelworm.

Two plants of each of 171 resistant clones were grown in 4-inch pots of soil containing 40 eggs/gm and forming two series (Series I and II). Fifty plants of a resistant clone comprised Series III and ten plants of the commercial variety, Kerr's Pink, were used to follow the development of cysts on roots. The pots were sunk to the rim in the ground.

After ten weeks, when the cysts on Kerr's Pink were yellow or orange in colour, the resistant plants were removed from the pots and cysts attached to the external root mats in an exposed position were counted. The results are given in Table I.

It can be deduced from Table I that when one or more cysts occurred on any plant in Series I and II combined, the probability of detecting one or more cysts on the other plant of the pair (P = 4 %) was less than that of detecting one or more cysts on a plant chosen at random from either series (P = 7% and 10%). Since there was no tendency for cysts to occur on pairs of plants it was concluded that the clones did not differ in their susceptibility to "occasional" cysts.

Table I showed, that the level of resistance to the Boghall population conferred

TABLE I

The incidence of cysts on test and control plants

Class	0	1	2	7	10	40	Totals	Failed
Frequencies, test plants								
Series I	151 92 %	11 6.7 %	1 0.7 %	0	0	0	163	8
Series II	141 89.8 %	12 7.6 %	2 1.3 %	0	1	1	157	14
Frequencies, control plants								
Series III	44 95.6 %	1 2.2 %	0 0 %	1	0	0	46	4
Expected frequencies Poisson distribution	90 %	9.5 %	0.1 %					

Class	* 0,0	0,1	0,2	0,10	1,40		
Frequency of pairs of results from Series I and II	124	21	2	1	1	149	22

* 0,0 = no cysts observed on either plant of a pair, 0,1 = none on one and one on the other and so on.

by the gene *H* could be specified accurately as the level of resistance which resulted in 90 % of the infected plants remaining cyst-free. Therefore, if all the biotypes in the Boghall population were uniform in pathogenicity towards plants of genotype *H*, or if aggressive biotypes were uniformly distributed throughout the population as a whole, then the percentage frequencies of plants bearing no cysts, one cyst, two cysts and so on should fit the hypothetical Poisson distribution. If the occurrence of 7, 10 and 40 cysts on three of the resistant plants is omitted, agreement is reasonable. Probably the pots in which these three plants were grown had contained at least one cyst which had given rise to a family of aggressive larvae.

The level of resistance determined above was regarded as the "standard" level of resistance to aim at in new varieties of potato. Thus 90 % of the plants of a clone or variety possessing standard resistance remain cyst-free on the basis of an inspection of soil-balls after exposure to infection from cysts in 4-inch pots containing a culture medium of 40 eggs/gm infectivity. The incidence of cysts on the remaining 10 % is immaterial to this definition. The type of culture medium in use at the Scottish Plant Breeding Station is prepared by mixing air-dried, infested soil of particle size less than 0.1 inches with coarse sand. The volume of sand required to reduce infectivity to 40 eggs/gm is usually 10-15 times the volume of soil. The pots are sunk to the rims in an electrically heated sand plunge. This method of sand culture superseded the soil culture in use at the time of the investigation reported above. The change in conditions has had no appreciable effect on the incidence of "occasional" cysts on plants of genotype *H*.

Scale of resistance to eelworm

Clones	*resistance*	*sign*	
Group 1	above standard	—	all plants of a clone have cyst-free soil-balls.
			90 per cent of the plants of a clone have cyst-free soil-balls.
Group 2	near standard	±	
			Mean count of 10 cysts per soil-ball (equivalent to a susceptibility index of 2 cysts per 100 windows-with roots).
Group 3	below standard	+	
			250 cysts per 100 windows-with-roots, maximum recorded susceptibility index.

Standard resistance is the first of two intermediate points in the accompanying, continuous scale of resistance to eelworm. A mean count of ten cysts on the plants of a clone is the second point. The latter point was selected (a) because it was considered that clones in group 2 might be useful for breeding purposes

in certain instances, particularly if the near standard resistance were controlled by a single gene and (b) because group 2 (±) created a marked discontinuity between group 1 (—) and group 3 (+). For tabulating strain relationships (Table IV) the minus sign means "resistant" or "non-aggressive" depending on the point of view and the plus sign means "susceptible" or "aggressive". The ± sign indicates an intermediate or doubtful strain reaction. Group 3 covers a very wide range of resistance as reflected by the difference between a mean count of 10 cysts and the recorded maximum of 1,000-1,250 cysts observed to date over the surface of a 4-inch soil-ball under the conditions appropriate to the assessment of standard resistance. The perforated can method (DUNNETT, 1957) is used to assess variation in level of resistance within group 3 and counts of 10 cysts or more are expressed in the form of a susceptibility index.

THE RESISTANCE TO EELWORM OF SOLANUM VERNEI, S. SANCTAE-ROSAE AND S. MULTIDISSECTUM

DUNNETT (1960) concluded that the resistance of *S. vernei* to both the Duddingston and Boghall populations was above standard and controlled by a system of polygenes.

Resistance to the Duddingston population in *S. multidissectum* and *S. sanctae-rosae* was detected in 1956 (DUNNETT, 1958). The progeny of *S. sanctae-rosae* (P. H. 328) comprised four seedlings free from cysts and one seedling bearing one visible cyst. The progeny of *S. multidissectum* (P.H. 1366) comprised five seedlings free from cysts and one seedling which supported six visible cysts. More than 200 cysts were visible on the control plants under the same conditions. Subsequently, three cyst-free seedlings were selected from each progeny and were grown on to the yield tubers. The selected seedlings were now clones and crosses were made between clones of the same species. In 1957 up to six plants of each clone were tested against the Boghall population and an equal number were tested against the Duddingston under the conditions already laid down for assessment of standard resistance. The gene *H* from subsp. *andigena* C.P.C. 1673, was present in the resistant, control clone 71/1 but not in the susceptible control clone 71/17 which was about equally susceptible to both populations.

The susceptibility indices recorded are given in Table II together with the number of cysts on the root mat, if ten cysts or fewer were present. Resistance in the three clones of *S. sanctae-rosae* to both populations was above standard. The resistance of the three clones of *S. multidissectum* to the Duddingston and Boghall populations was above standard and below standard, respectively. The Boghall population was less aggressive towards *S. multidissectum* than was the Duddingston population towards the control clone incorporating the gene *H*. Judging from the susceptibility indices of the susceptible control clone, conditions seem to have been more conducive to cyst formation in the Duddingston series.

In 1957, to compensate for the shortage of clonal material, 100 seedlings of

TABLE II

Susceptibility indices of clones; cysts per 100 windows-with-roots.
(a) *Boghall population.* (b) *Duddingston population.*

Control clone (genotype H)							
71/1	(a)	0(F)	0(F)	0(F)	0(F)	0(F)	0(F)
	(b)	72	82	93	103	106	123
S. multidissectum							
clone 14/2	(a)	3	3	3	5	6	–
	(b)	0(F)	0(F)	0(F)	0(F)	0(F)	0(F)
clone 14/4	(a)	0(2)	0(2)	2	2	4	5
	(b)	0(F)	0(F)	0(F)	0(F)	0(F)	0(F)
clone 14/5	(a)	0(4)	3	4	10	14	14
	(b)	0(F)	0(F)	0(F)	0(F)	0(F)	0(F)
S. sanctae-rosae							
clone 4/2	(a)	0(F)	–				
	(b)	0(F)	0(F)				
clone 4/4	(a)	0(F)	0(F)	0(F)	0(F)	–	–
	(b)	0(F)	0(F)	0(F)	0(F)	0(F)	0(F)
Control clone (genotype h)							
71/17	(a)	72	82	93	102	106	123
	(b)	150	153	180	199	237	245

(F) = free from cysts: (1, etc.) = cysts observed over whole soil-ball surface.

S. sanctae-rosae and 100 seedlings of *S. multidissectum* were tested for resistance to the Boghall and Duddingston populations. Table III records the distribution of these seedlings and plants of the control clones, according to the estimated number of cysts observed over the surfaces of soil-balls from 3-inch pots. The results supplement those of Table II.

In 1958, a distinct segregation of resistance to the Duddingston population was apparent in a progeny of parentage *S. multidissectum* (unreduced gametes) x *S. tuberosum*. Ten of the seedlings remained free from cysts, one seedling supported one cyst, and the remaining seedling had a suspectibility index of 24 cysts per 100 windows-with-roots. The Duddingston population multiplied 5.6 times on the last seedling and declined by four-fifths in pots with the other seedlings.

In 1959, there was evidence of undiminished resistance in the first backcross of the hybrids with *S. multidissectum* to *S. tuberosum*. Resistance was dominant and by replicating some of the first backcross families it may be possible to establish whether there is complete discontinuity between the resistant and susceptible classes.

Also in 1959, there was evidence of polygenic inheritance of resistance to the

TABLE III

Distribution of seedlings according to cyst count in progenies divided between the Boghall and Duddingston populations.

Species and control clones	Number of cysts								
	0	1-2	3-5	6-11	12-24	25-49	50-99	100-200	200+
	Progenies infected by Boghall population								
S. sanctae-rosae	45	0	0	0	0	0	0	0	0
S. multidissectum	0	10	5	11	4	4	7	0	0
71/1 (genotype H)	8	0	0	0	0	0	0	0	0
71/17 (genotype h)	0	0	0	0	0	0	0	6	2
	Progenies infected by Duddingston population								
S. sanctae-rosae	43	0	0	0	0	0	0	0	0
S. multidissectum	47	3	0	0	0	0	0	0	0
71/1 (genotype H)	0	0	0	0	1	1	5	1	0
71/17 (genotype h)	0	0	0	0	0	0	0	8	0

Duddingston population in crosses between *S. sanctae-rosae* (4n) and *S. tuberosum,* although the hybrids appeared to be less uniform in resistance than the hybrids between *S. vernei* (4n) and *S. tuberosum.*

In resistance *S. multidissectum* can be classed with *S. tuberosum* subsp. *andigena,* clone C.P.C. 1673. Both species are sources of the standard level of resistance to certain specified populations of the eelworm only, and the resistance is conferred by one or few genes. The resistance of subsp. *andigena* C.P.C. 1673 is not dependent on the failure of larvae to hatch at a normal rate in the vicinity of the root systems of the resistant plants (WILLIAMS, 1957), and the same is apparently true of the resistance in *S. multidissectum.* The latter species is indigenous to Peru und Bolivia, which is also the known distribution of the eelworm in South America (BAZAN DE SEGURA, 1952; BELL & ALANDIA, 1955). There are no cultivated varieties of *S. multidissectum* but many varieties of subsp. *andigena* are cultivated in the Andean highlands including parts of Peru and Bolivia, where the clone C.P.C. 1673 was collected. A Peruvian population of the eelworm proved to be aggressive towards plants having the resistance of C.P.C. 1673 (QUIVEDO DÍAZ, SIMON & TOXOPEUS, 1956) and was, therefore, akin to the Duddingston population in pathogenicity. *S. multidissectum* possesses the standard level of resistance to the Duddingston population.

These facts suggest that a type of resistance to eelworm may be advantageous to a species of potato exposed to eelworm attack and that pathogenic adaptation in the eelworm will tend to negate the advantage eventually.

S. sanctae-rosae can be classed with *S. vernei,* in that the resistance of both species appears to be polygenic and proof against known variation in the patho-

genicity of the eelworm. WILLIAMS (1956) noted that the resistance of *S. vernei* was associated with a lower rate of hatching than was the resistance of subsp. *andigena* C.P.C. 1673, and fewer larvae entered the root system of the resistant plants. *S. vernei* and *S. sanctae-rosae* may occupy a position at the fringe of the eelworm's host range, a position which perhaps owes more to the process of speciation within the tuber-bearing section of the genus *Solanum* than to natural selection for resistance to eelworm. It may be significant that *S. vernei* and *S. sanctae-rosae* are limited in distribution to Argentina (HAWKES, 1956), where the eelworm is not yet reported to occur.

STRAIN RELATIONSHIPS

In theory, *Heterodera rostochiensis* may be subdivided as follows:

(a) populations containing few or no aggressive biotypes.
(b) populations composed entirely or almost entirely of biotypes characterised by a particular type of aggressiveness, negating a particular type of resistance.
(c) populations intermediate between (a) and (b) or intermediate between populations of different pathogenicity classed under (b).

Group (a) is the non-aggressive strain of the eelworm. Group (b) comprises the aggressive strains, each of which has a selective host as previously defined. A population in group (c) is representative of no particular strain but may be regarded as a mixture of strains. Such a mixture may be resolved in the direction of a breeders' strain of the eelworm by selective culture on a selective host, or the mixture may be perpetuated by non-selective culture on a neutral host, in contradistinction to a selective host.

Group (a) in Table IV is hypothetical and must remain so until a representative population is obtained direct from the field, since by definition the non-aggressive strain cannot be cultured selectively. Group (b) is represented by the Boghall strain, which will be selectively cultured in future on plants having the resistance of *S. multidissectum* in an effort to increase the aggressiveness of this strain. Group (b) also includes the Duddingston strain which already has a history of selective culture, expressed by writing Duddingston x 4 C.P.C. 1673, and which multiplies from 20 to 30 times on plants having the resistance of subsp. *andigena* C.P.C. 1673. The pathogenicity of the Duddingston population is the antithesis of the pathogenicity of the Boghall population in that neither population contains more than a negligible proportion of biotypes capable of surviving to female maturity in the selective host of the other population. The selective culture of Duddingston x 4 C.P.C. 1673 may have been partially responsible for this relationship.

Group (c) in Table IV is represented by a mixture of the Boghall and Duddingston strains. Such a mixture has already been multiplied on a neutral host and the resulting population will be selectively cultured on plants possessing the combined resistance of subsp. *andigena* C.P.C. 1673 and *S. multidissectum*. The objective is the formation of a new strain which will negate the genetic combination

TABLE IV

Tabulation of strain relationships

Strain of *H. rostochiensis* \ Differential host Series	Clone 71/17 "universal host"	subsp. *andigena* C.P.C. 1673 clone 71/1, genotype H	*S. multidissectum* clone 14/5	*S. multidissectum* × clone of genotype H
a Non-aggressive	(+)	(—)	(—)	(—)
b Boghall	+	—	+	?
Duddingston × 4 C.P.C. 1673	+	+	—	?
c Boghall + Duddingston × 4 C.P.C. 1673	+	+	+	?

+ resistance below standard

— resistance standard or above standard

of both types of resistance. The search for a new type of resistance can then begin.

S. vernei and *S. sanctae-rosae* are excluded from Table IV because hybridisation with *S. tuberosum,* the first step in breeding, gives diminished resistance due to polygenic inheritance and there seems little possibility of establishing the relationship between strains and designated genes in these species.

The range of genetic variability in naturally occurring populations is probably greater than in selectively cultured strains descended from relatively few aggressive biotypes. By using strains of minimum variability, perhaps even strains descended from a single aggressive female, the breeder can hope to distinguish with the greatest possible precision between kinds of resistance to potato root eelworm in the many species of potato indigenous to South and Central America.

Unless immunity from potato root eelworm or a type of resistance which always suppresses cyst formation can be incorporated in new varieties of potato, the problem of controlling the eelworm by varietal resistance is a problem of strains. Separate infestations can be treated as separate problems. The answer to these problems is an appropriate rotation of different resistant varieties at suitable

intervals in particular fields. Although there appears to be no lack of resistance in the tuber-bearing Solanums it may be many years before a range of different types of resistance is available to commercial varieties of potato.

ZUSAMMENFASSUNG

Stämme des Kartoffelnematoden (Heterodera rostochiensis *Woll.*)

Eine selektive Wirtspflanze für eine aggressive Population des Kartoffelnematoden ist ein resistenter Klon oder Stamm, an dem sich lebensfähige Biotypen entwickeln, was dem normalen Typ des Kartoffelnematoden nicht gelingt. Die Neigung zu einem homozygoten Angriffsvermögen in einer Population steigert sich bei einem selektiven Wirt von Jahr zu Jahr. Verschiedene Stämme haben verschiedene selektive Wirte, die zusammen eine unterschiedliche Wirtsserie bilden. Es wird vorgeschlagen, diese auf Pflanzen zu beschränken, die wenigstens ein Standardmaß von Resistenz gegenüber mindestens einem Stamm besitzen, um ein praktisch brauchbare Klassifizierung zu ermöglichen. Das Normalmaß der Resistenz (Standard) wird als dasjenige bestimmt, das sich aus 90 % Pflanzen eines Klons ergibt, die nach Infektion unter bestimmten Bedingungen zystenfreie Wurzelballen haben. Die Resistenz wird eingeteilt in „übernormal" ($>$ 90 % Pflanzen zystenfrei = nichtaggressiv), „normal" (90 % zystenfrei) und „unternormal" ($<$ 90 % Pflanzen zystenfrei = aggressiv). Eine mittlere Zahl von 10 Zysten je Wurzelballen ist die obere Grenze für die „Normal"-Resistenz. Die Beziehungen zwischen der Resistenz von subsp. *andigena* C.P.C. 1673, *S. multidissectum* und den aggressiven Populationen Duddingston und Boghall sind tabellarisch niedergelegt. Ein hypothetischer nichtaggressiver Stamm wird vorausgesetzt. Gewisse Ähnlichkeiten zwischen der Resistenz und Verteilung von subspec. *andigena* und *S. multidissectum* auf der einen Seite und *S. vernei* und *S. sanctae-rosae* auf der anderen Seite werden besprochen. Durch Arbeiten mit Nematodenstämmen von minimaler genetischer Variabilität kann der Züchter hoffen, zwischen Arten von Resistenz mit größerer Genauigkeit zu unterscheiden. Wenn nicht Immunität oder ein gewisses Maß von Resistenz, die immer Zystenbildung unterdrücken, in Handelssorten der Kartoffel verkörpert werden kann, ist das Problem der Bekämpfung der Nematoden durch Sortenresistenz ein Problem der Nematodenstämme.

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RESISTANCE OF WHITE CLOVER VARIETIES TO THE CLOVER CYST-EELWORM, *HETERODERA TRIFOLII* GOFFART

BY

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A monosexual strain of clover cyst-eelworm *Heterodera trifolii* Goffart is widespread in meadows in the Netherlands (OOSTENBRINK, 1957) and may be one of the causes of poor growth of white clover. This paper describes a search for resistance in white clover varieties. One hundred clones selected for good growth and other desirable characteristics under Dutch conditions were supplied by the Foundation for Agricultural Plant Breeding (S.V.P.), Wageningen. They originated from the commercial varieties listed in Table I.

In the spring of 1958, cuttings were taken and planted in 500 ml pots containing a well-mixed, heavily infested soil from a broken-up meadow. The mean infestation was 940 eggs/100 ml soil. The external root-mats were examined at 15, 21 and 27 weeks from planting. When the root-mat became too dense for reliable examination, new cuttings were taken from those clones on which few or no cysts had been found and these were examined again at 13 and 22 weeks from re-planting.

In the spring of 1959 new cuttings were taken from clones showing evidence of resistance and potted as before in five-fold replication. The root-mats of these plants were examined twice during the growing season.

Of the 100 clones tested in 1958, sixty-one were heavily attacked, twenty-five were lightly attacked, eight appeared to be without cysts and six could not be judged with certainty. These six showed either poor root growth or a patchy infestation. Of the thirty-nine clones re-tested in 1959, fifteen had 25 or more cysts per rootball, twelve had 5-25 cysts per rootball, eight had 1-5 cysts per rootball and four clones died.

These results show that all clones successfully tested developed one or more cysts on the roots: none was absolutely resistant. On the well rooted plants of the eight lightly infested clones, very few cysts were formed in 1958 or 1959 while susceptible plants bore very many. The position is comparable with that found in the breeding of potatoes resistant to *Heterodera rostochiensis* Wollenweber (HUYSMAN 1957) and it may be concluded that the forementioned clover clones are virtually resistant.

It was surprising that several resistant clones should occur among the small

TABLE I

Origin of clones tested and their classification

Country	Variety	Number of clones infested with *H. trifolii*			
		Practically free	Slightly infested	Heavily infested	Died
Australia	Victory	—	—	2	1
Denmark	Lodi Otofte	2	2	26	1
	Pajberg Grasmark	—	—	1	—
Finland	Tamisto	—	—	2	—
Germany	N.F.G.	1	—	5	—
Gt. Britain	Kersey	1	—	6	—
	S 100	1	—	—	—
Hungary	—	1	—	1	—
Italy	Ladino	—	1	1	—
Netherlands	Sel. S.V.P.	—	3	3	1
	Cultuurklaver C.B.	—	1	6	—
	Weideklaver C.B.	—	—	3	—
	Wikla	—	—	3	—
	Inlandse Witte	—	1	—	—
	Landras Fries-Gronings	—	—	5	1
New Zealand	Certified motherseed	1	2	1	—
Sweden	Nora	—	—	7	—
	Hero	—	—	2	—
U.S.A.	Ladino	1	4	2	—
	Totals	8	14	76	4

number tested. This might be due to a kind of natural selection that had taken place before the clones were tested. The arable land on which they were grown was heavily infested with *H. trifolii*. Because this strain might be different from the one prevalent in meadows, the cuttings were carefully freed from roots and soil before planting in the meadow soil. In Table I the results are classified according to country of origin. Resistant clones have come from seed originating in six different countries.

ZUSAMMENFASSUNG

Resistenz von Weisskleesorten gegenüber dem Kleezystenälchen, Heterodera trifolii *Goffart*

Hundert Weissklee-Stämme aus verschiedenen Ländern wurden auf Resistenz gegen das Kleezystenälchen, *Heterodera trifolii* Goffart untersucht. Es handelt sich hier um einen monogenetischen Nematodenstamm, der ziemlich allgemein in Wiesen vorkommt und Weissklee schädigt. In Topfversuchen der Jahre 1958 und 1959 wurden 8 Kleestämme verschiedener Herkunft gefunden, die bei guter Entwicklung nur 1-5 Zysten pro Topfballen aufzeigten, während an empfindlichen Pflanzen einige hundert Zysten gefunden wurden. Erstere sind deswegen als praktisch resistent zu betrachten.

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THE INFLUENCE OF DIFFERENT ROTATIONS ON A POPULATION OF PEA CYST EELWORM, *HETERODERA GÖTTINGIANA* LIEBSCHER

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The range of crops susceptible to *Heterodera göttingiana* is limited in practice to peas, *Pisum sativum,* field beans and vetches, *Vicia* spp., (JONES 1950, OOSTENBRINK 1951, WINSLOW 1955, JONES & MORIARTY 1956, BROWN 1958). For this reason it might be expected that crop rotation could be used as a control measure as with other species of *Heterodera.* However, heavy infestations and pea failures have been recorded in fields where peas or other hosts have not been grown for many years. To obtain more information about field populations the following experiments and population studies were made.

CROP ROTATION EXPERIMENT

Procedure

In 1948 a field on heavy clay soil in the province of Zeeland was selected. This field showed a fairly uniform, heavy infestation of *H. göttingiana* after growing peas in the years 1948 and 1949. Other cyst-forming nematodes were not present.

From 1950 to 1957, six different rotations were followed: peas every year; peas once every two years; three years; four years; five years and six years. In 1958 peas were grown on the whole field again. The non-host crops in the experiment were cereals or sugar beet, depending on the year. Replication was four-fold. Two replicates received a complete fertilizer, and the other two both dung and fertilizer. In presenting the results the four replicates were finally taken together since manuring did not cause notable differences. The degree of infestation of each plot was determined yearly before planting and expressed per 100 ml of air-dried soil. The yield of the peas was determined by weighing seed and haulm after harvesting.

Results

Fig. 1 shows the means of results from the four replicates for each year of each of the six rotations; yield of peas above the line and number of viable cysts below.

The results suggest a negative regression of yield on initial eelworm population. This is less obvious for different years than when the effects of different rotations

are compared within the same year. This is shown in Fig. 2 for the year 1957 and for the final year 1958, when a pea crop was grown on the whole trial field. Fig. 1 also shows the remarkable fact that on all plots on the field the populations of pea cyst eelworm decreased from 1951 to 1955 whether peas or other crops

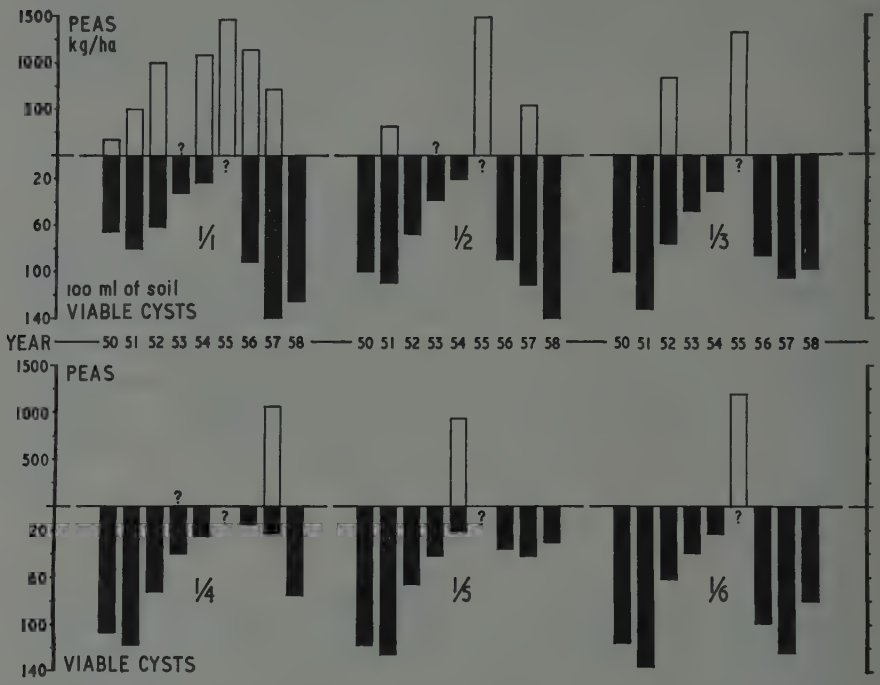

Fig. 1. Initial pea cyst-eelworm populations and yield of the subsequent pea crops in successive years for six different rotations (peas every year to one year in six). Yield of peas above the line, viable cyst populations below. Means of four replicates.

were grown. In 1955, however, when peas were grown the infestation level rose considerably, even on plots growing peas only one year in six, but this did not occur when peas were grown in 1954 on plots having peas once in five years.

In 1956, 1957 and 1958 great differences in levels of infestation were observed which appeared to be due to the efficient nematode reproduction on the 1955 pea crop (in peas one year in four, the 1957 crop was responsible). The differences did not appear to be attributable to crop growth because the yields of peas from the crop in 1955 on the one in six rotation plots and that in 1954 from the one in five were similar, 1197 and 945 kg/ha respectively. Both crops grew well initially but became diseased later in the season. This is also borne out by comparing the cyst numbers in 1958 on the replicates with peas one year in six (peas in 1955) with those on the one-in-five plots (peas in 1954). The former, 146 cysts, was about double the latter—78 cysts.

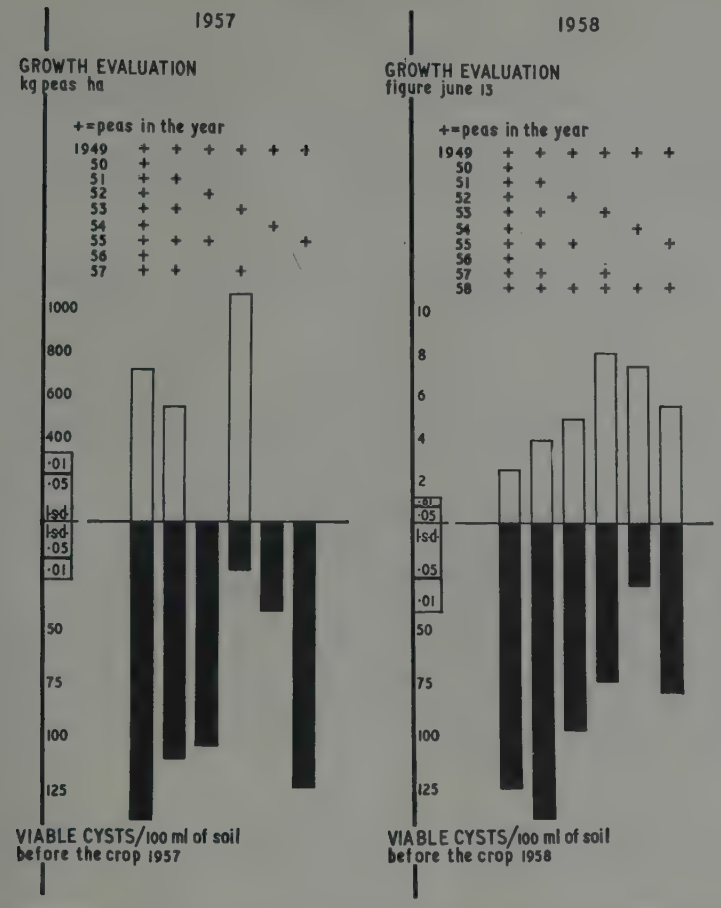

Fig. 2. Evaluation of the effect of pea cyst-eelworm population on the yield growth of peas in the years 1957 and 1958 on plots subjected to different rotations. Yield or growth of peas above the line, initial viable cyst populations below. Means of four replicates.

CONCLUSION

From these results reproduction of *H. göttingiana* seems to differ widely from one pea crop to another and pea crops on which poor reproduction occurs are fairly common. The rate of decrease in population under non-susceptible crops seems to be fairly slow. A year in which reproduction is efficient on peas may, therefore, decide the degree of infestation for several years.

In this trial field, it was not possible to harvest a good crop of peas with any of the rotations tried, even under favourable conditions of treatment and manuring. The yield of peas was never more than half that expected on an uninfested soil.

ZUSAMMENFASSUNG

Der Einfluss verschiedener Fruchtfolgen auf eine Population von Heterodera göttingiana *Liebscher*

Auf Grund der in Fig. 1 dargestellten Befunde in einem Versuchsfeld auf schwerem Boden mit hoher Anfangsverseuchung von Erbsennematoden (*Heterodera göttingiana*), wurden die Veränderungen der Nematodenpopulationen unter dem Einfluss von sechs verschiedenen Fruchtfolgen mit mehr oder weniger intensivem Erbsenbau diskutiert.

Der Einfluss vergleichbarer Erbsensaaten hinsichtlich der Nematodenvermehrung ist im Jahre 1955 sehr viel grösser gewesen als in den Jahren 1950-1954. Die Verseuchungsstufe eines Feldes scheint stark abhängig zu sein von der Fähigkeit der letzten Erbsensaat, die Anzahl der Nematoden zu steigern.

In keinem Versuchsjahr und bei keiner der angewandten Fruchtfolgen (1/1 bis 1/6 Erbsen) konnte auf dem verseuchten Feld mehr als eine halbe Ernte gewonnen werden, trotz guter Versorgung und guter Düngung.

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PERIODICITY IN SPONTANEOUS HATCHING OF *HETERODERA ROSTOCHIENSIS* IN THE SOIL

BY

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Decrease in populations of *Heterodera rostochiensis* Woll. in soil has been considered as a continuous process and natural enemies have been thought to be responsible for the decline of larval numbers within the cysts (OOSTENBRINK 1950). The following observation suggested a different explanation.

In a field of sandy soil, seventy-two squares of 1 m^2 each were sampled on 22 May 1956 and 9 October 1956. In each of these samples numbers of larvae of *H. rostochiensis* within cysts and free in the soil were counted. The results (Table I) showed two striking features: a high number of larvae free in the soil in spring and no decrease of numbers of larvae (eggs) within the cysts between May and October. This suggested that the decrease in populations of *H. rostochiensis* in the soil in the absence of a host plant was a matter of spontaneous hatching probably during a short period in spring. In the following two years this hypothesis was tested.

TABLE I

Numbers of cysts, encysted eggs and free larvae of Heterodera rostochiensis *in the soil per 5 g of soil.*

	Spring 1956	Autumn 1956
Cysts	2.1	2.2
Encysted eggs	109 ± 3.3	105 ± 3.3 *
Larvae in soil	34.5	0.25

* L.s.d. 5% point 10; 1% point 15.

FREE LARVAE IN THE SOIL

The occurrence of free larvae in the soil was investigated in a field of sandy soil at Wageningen within which two pairs of plots were set up so that, in 1957 and again in 1958, one of each pair had grown potatoes two years previously and the other one year previously.

In this way the behaviour of predominently one-year old and two-year old eelworm populations could be compared. During the experiment the plots were kept fallow. Within each plot fixed metre squares were sampled between March

and September. From each square eighty cores of 20 g of soil were taken and, after mixing, the nematodes were extracted from a 500 g aliquot by means of a Seinhorst elutriator.

The results of the larval counts are given in Fig. 1, A and B. To make comparison easier, the numbers of larvae free in the soil at different dates are expressed as percentages of the encysted population before appreciable numbers of free larvae began to appear. In the first year (Fig. 1, A), the count made on the first sampling date was used as the basis for the percentages. In the second year (Fig. 1, B and C), two estimates of the encysted population were made before appreciable numbers of larvae appeared in the soil and so the numbers of free larvae are expressed as percentages of the mean of these two.

In the first year, a sample from the plots which grew potatoes in 1955 and 1956 taken on 14 February was also investigated on 14 March after having been kept in the laboratory at room temperature. The results are given in two columns in Fig. 1 A.

In the second year (1958), soil from the plots which grew potatoes in 1956 and 1957 was placed in separate containers in the laboratory at 15° C on 27 March. When the field plots were sampled a sample was also taken from each of these containers. Numbers of larvae found in these soils are presented graphically in Fig. 1 C, expressed as percentages of counts of encysted eggs on 27 March.

DECREASE IN NUMBERS OF LARVAE WITHIN CYSTS DURING SPRING AND SUMMER

In the first year the encysted populations were only determined for samples taken on the first and last sampling dates. In the second year, they were determined for all sampling dates. The results of the determinations in autumn and spring are given in Table II. The decline in the summer of the second year is presented graphically in Fig. 1 D.

TABLE II

Decrease of cyst contents in 1957 and 1958

Last potato crop in	Spring 1957	Autumn 1957		Decreases % since spring	
1955	603	284		53	
1956	8311	3350		60	
	Spring 1958	Autumn 1958	Spring 1959		
1956	4055	2835	2479	30	39
1957	14695	8665	9064	41	38
After HUYSMAN (1957):					
	Autumn 1954	Autumn 1955			
1953	2560	2255		11	
1954	20560	13540		34	

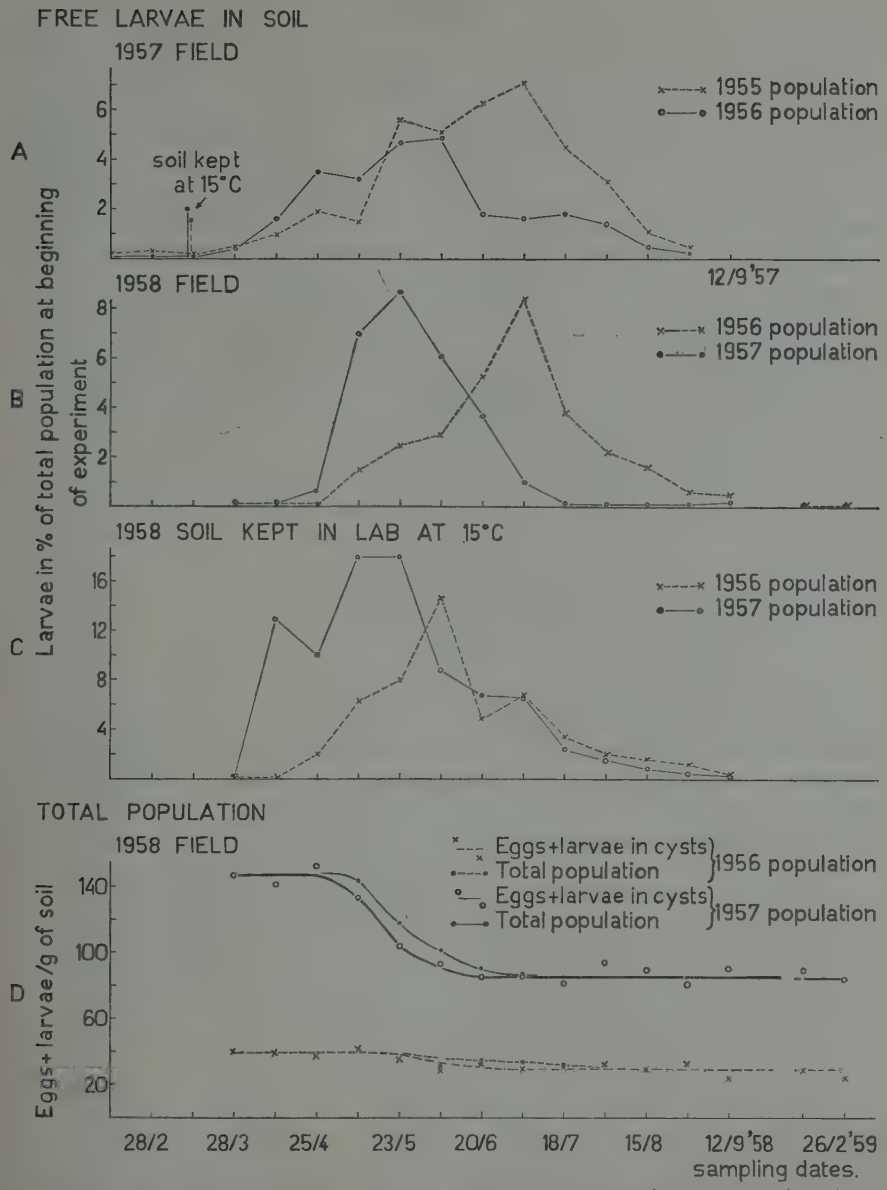

Fig. 1A. Field results in 1957 for plots which grew potatoes in 1955 and 1956; B. Field results in 1958 for plots which grew potatoes in 1956 and 1957; C. Results from soil kept in the laboratory at 15°C. Soil taken in 1958 from plots growing potatoes in 1956 and 1957; D. Field results, changes in cyst contents during 1958 for 1956 and 1957 populations. Total population equals encysted population plus larvae free in soil. Each point in the graphs is the mean of estimates from five metre squares.

DISCUSSION

In both 1957 and 1958 the number of free larvae of *H. rostochiensis* in the soil increased in spring or early summer and then decreased again to a very low number (Fig. 1 A and B). In the soil with the 1 year old populations the increase was rather rapid. In both years there were high numbers of larvae in the soil in May and the beginning of June. In the soil with the 2 years old populations the maximum number of larvae was found 6 weeks later and relatively high numbers of larvae were found in the soil during a somewhat longer period than with the 1 year old population. In both years spontaneous hatching started earlier in soil kept in the laboratory at room temperature or at 15° C than in the field.

Indoors the difference in time between the dates of maximum hatching for the populations of different ages remained but was smaller than out of doors. Probably the higher temperature stimulated the larvae but also the loosening of the soil may have had something to do with it.

A considerable decrease in cyst contents in the 1 year old population (Fig. 1 D) appeared to coïncide with the period of increasing numbers of larvae free in the soil (Fig. 1 A, B). The cyst contents remain constant during the remainder of the year.

It is not possible to indicate exactly the period of decrease in cyst content of the 2 year old population of Fig. 1 D.

Decline of populations in *H. rostochiensis* seems largely due to spontaneous hatching during 6 to 12 weeks in spring or summer. Fig. 1 D shows that reduction between the beginning of June and the following March was almost negligible in the two cases investigated here. BIJLOO & BOOGAERS (1956) found the same. HESLING, 1958 found a similar phenomenon with *H. avenae* (= *major*). However the period of decrease of cyst contents in the absence of a host plant was much longer here (March-September).

In both years the 1 year old populations decrease more than the 2 year old ones (Table II). This is in accordance with the results of HUYSMAN (1957).

With the exception of the data shown in Table I the numbers of larvae found free in the soil are only a small proportion of the total population. Actually the sum of the larvae found on the different sampling dates is more or less of the same order as the decrease of the number found in the cysts between early spring and late summer. This might indicate that the average life of a larva after hatching was not much more than two weeks. Also the rather rapid decline of numbers of larvae in the soil at certain periods (Fig. 1 A, B) suggests a high mortality rate.

To investigate the duration of life of free larvae in the soil one, 2 and 3 year old cysts were collected from field soil in May and put in small bags of nylon gauze. These bags were placed in moist soil. After 3 weeks large numbers of larvae were found in the soil. The bags with the cysts were then removed and numbers of larvae in the soil were determined at regular intervals afterwards.

During the first weeks there was hardly any decrease but after 7 weeks about half of the larvae had died. The remainder had become light in colour most probably because of loss of reserve food. There was no difference between the larvae from cysts of different ages. So the results of this experiment do not support the hypothesis that larvae of *H. rostochiensis* have a short life in the soil after having hatched spontaneously.

The cyst content may decrease periodically through other causes than spontaneous hatching. However the coïncidence of periodic decrease of cyst contents and the presence of larvae in the soil, together with the absence of a workable hypothesis based on other causes for the decrease of cyst contents strongly suggests that periodic spontaneous hatching is the chief cause for the decline of population numbers of *H. rostochiensis* in this soil.

However, to account for the difference between the number of larvae which left the cysts and those found in the soil, further investigations need to be done to determine what happens to the larvae in the soil under different conditions.

Thanks are due to Miss N. GRETMANN and Miss N. SCHUURMAN for technical assistance.

ZUSAMMENFASSUNG

Periodizität bei spontanem Schlüpfen von Heterodera rostochiensis *im Boden*

In einem Maisfelde wurden Mitte Mai 1956 sehr große Mengen spontan geschlüpfter Larven des Kartoffelälchens *Heterodera rostochiensis* Woll. gefunden. Im Oktober waren fast alle Larven wieder verschwunden. Der Inhalt der Zysten war jedoch noch derselbe wie im Mai; während der Sommermonate waren also fast keine Larven spontan geschlüpft.

Untersuchungen hierüber in den Jahren 1957 und 1958 ergaben daß tatsächlich die Zahlen freier Larven in Böden ohne Kartoffeln im Frühling schnell ansteigen und dann ziemlich schnell wieder absinken. Mit zweijährigen Populationen wird die maximale Zahl etwa sechs Wochen später erreicht als mit einjährigen Populationen. Der Zysteninhalt war zur Zeit dieses Maximums bis zum niedrigsten Wert gesunken und blieb bis März des nächsten Jahres unverändert.

In einem Laborversuch über die Lebensdauer von spontan geschlüpften Larven waren 50 % der Larven 6 Wochen nach dem Schlüpfen abgestorben. Diese Zahl stimmt nicht mit der aus den Feldwahrnehmungen berechneten Lebensdauer der Larven im Boden von etwa zwei Wochen überein. Dennoch weist die Koïnzidenz der Abnahme der Zahl der Eier in den Zysten und einer Zunahme der Zahl der Larven im Boden darauf hin, daß die Abnahme des Zysteninhalts in Abwesenheit einer Wirtspflanze durch spontanes Schlüpfen während einer kurzen Zeit im Frühjahr erfolgt.

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PROGRESS IN THE STUDY OF THE PHYSIOLOGY OF THE HATCHING FACTOR OF THE POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

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Concentrates of potato root diffusates possess cardiotonic activity and they may therefore be assayed by means of an isolated frogheart preparation (ELLENBY & GILBERT, 1957a). This finding is consistent with the view of Todd and his co-workers that the hatching factor of the potato-root eelworm contains an unsaturated lactone ring as an essential part of its structure (MARRIAN, RUSSELL, TODD & WARING, 1949). But the evidence for the latter belief is not decisive: moreover, as the factor has not yet been obtained pure, there is always the possibility that our results might be due to accompanying substances, that is, to impurities associated with the hatching factor. Although it is still true that our subsequent results may also be due to accompanying impurities, it is also true that the lactone hypothesis can still be maintained.

In addition to the fact that root diffusate concentrates stimulated the hypodynamic frog heart, our earlier work showed that this activity could be "potentiated" by the cardiac glycoside strophanthin, and that its heart activity was also increased by traces of hydrogen peroxide. (ELLENBY & GILBERT, 1957a, b). Because we showed that traces of peroxide also increased the hatching activity of the concentrate it was suggested that the physiological mechanism by which the factor acted on heart and in hatching might be similar. But many substances influence the beat of the frog's heart and our experiments have therefore been planned from two points of view. Firstly, we have examined the possibility that particular substances other than the hatching factor, might be responsible for the cardiotonic activity. Secondly we have suggested the hypothesis that the activity is due to the affinities of the hatching factor with cardiac glycosides; if this is so, substances which influence the activity of the glycosides might also affect hatching factor activity.

The effect on the frog heart might be due to ionic contamination of our preparations. It is well known that the perfused heart is very sensitive to the ionic background, and a slight trace of calcium would be quite sufficient to explain the cardiotonic effect of our preparations. Since our early experiments we have been able to obtain root diffusate solutions of negligible ion content which never-

theless still influence the frog heart (ELLENBY & GILBERT, 1958). We have also determined the minimal quantity of calcium ion which had to be added to our low calcium perfusion solution to give a detectable effect. Knowing the conductivity of our hatching factor solutions, we have been able to decide whether, assuming it were all due to calcium ions, the cardiotonic activity was explicable on these grounds. There was much ionic material present in one preparation and, assuming this to be calcium, the heart test would lead to an overestimation of its hatching factor content; other types of test with this preparation confirmed his view. But

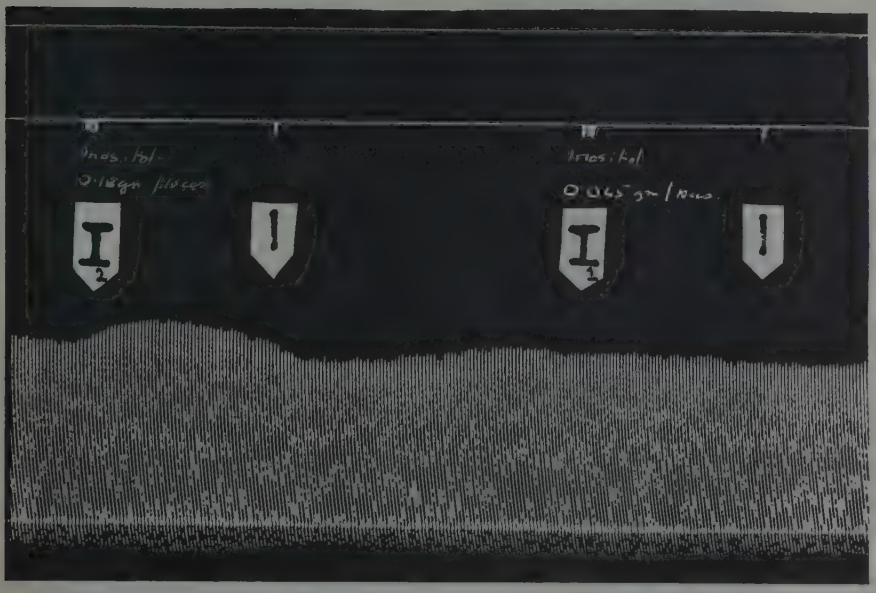

Fig. 1. Isolated frog heart preparation. All tracings read from left to right with heart first being perfused with low calcium Ringer. At signal (I_2) heart is perfused with inositol in low calcium Ringer (1800 mg./100 ml). No response is shown when the heart is perfused with a more dilute solution of inositol (I_1, 450 mg./100 ml). Each test solution is followed by low calcium ringer (!). For all tracings time signal, 20 sec.: Straub preparations.

for all the other preparations, ionic contamination could not explain the results.

WALLACE (1957) has shown that inositol and similar substances did not stimulate hatching of the beet eelworm: WOOD & SERRO (1954) had previously shown these substances to be present in the root diffusate of various plants. Fig. 1 shows our heart preparation tracing for pure inositol, a marked response being given at the concentration of 1800 mg/100 ml low calcium Ringer. But since, even with one of our weaker and very impure concentrates, a similar response is given at a concentration of 18 mg/100 ml, or at about one hundredth of the concentration for pure inositol, it is clear that this substance can safely be excluded.

The assumption about the chemical affinities of the hatching factor is supported by the fact that root diffusate may be assayed by colorimetric methods developed for cardiac glycosides (ELLENBY, 1958). And there is evidence that the hatching factor and cardiac glycosides may operate through a similar physiological process. SCHATZMANN (1953) and various later workers have shown that cardiac glycosides influence the active transport of ions and our findings (ELLENBY & GILBERT, 1958b, c) that the activity of the hatching factor is very sensitive to the ionic background is consistent with the view that ion movements are involved in the

Fig. 2. Isolated toad heart preparation. At signal (D), heart is perfused with 16 mg. of hatching factor preparation G_1 in 100 ml. low calcium Ringer, followed by low calcium Ringer (!).

hatching process. Moreover, the influence of various drugs—quinidine sulphate, veratridine, dinitro phenol, and sodium salicylate—on the activity of the hatching factor is also in reasonable agreement with the hypothesis. The view is also supported by the results of a single preliminary experiment with radioactive tracers, carried out with the help of Dr. Medd. Cysts were soaked in water containing $_{22}Na$. After treatment, half the cysts were placed in root diffusate and the other half in water. After four and five days eggs and larvae were examined by autoradiography. For the hatching factor treatment, there was evidence of radioactivity in 8 cases, and 3 with none, while for the water treatment there was evidence of activity in 3 cases, and 8 with none.

HAJDU has suggested (1957) that among substances having a cardiotonic effect, those with a lactone group affected the heart of the frog but not that of the toad, but those without this chemical group affected the hearts of both animals: ISSEKUTZ (1923) showed that toad heart is far less sensitive to cardiac glycosides than frog's heart. We found that although toad heart is less sensitive to hatching factor than frog's, the difference is not as great as anticipated on the lactone hypothesis. Fig. 2 shows that the minimal dose for a response from toad heart to one of the Groningen preparations was about 16 mg/100 ml: the minimal dose for a response to this preparation for frog heart is about 3 mg/100 ml. However, Hajdu's

Fig. 3. Isolated toad heart preparation responding to γ butyrolactone, 50 mg./100 ml. (L) followed by low calcium Ringer (!).

distinction needs modification for we found that the toad heart does respond to the simple lactones β propriolactone and γ butyrolactone (Fig. 3).

We have recently assayed a number of new concentrates from Dr. G. J. Janzen of Groningen, who had found that they stimulated hatching at very great dilution. With four of the preparations, we found that they stimulated the heart at dilutions at the levels similar to those obtained for pure cardiac glycosides, but that with more concentrated solutions there was no response. Fig. 4 shows, for one of these preparations, the positive responses obtained at 0.5 mg/100 ml and at 0.125 mg/100 ml: however, no response was obtained at 20 mg/100 ml. Results of this sort would be understandable if there were inhibiting substances also present: they might have a different threshold value from that of the hatching factor so that, on dilution, the action of the latter might have an opportunity of manifesting itself. We were interested to find that Dr. Janzen had formulated a similar view to explain some of her findings on hatch stimulation.

We have failed to obtain a heart response with one of Dr. Janzen's recent

preparations. It is unlikely, if inhibiting substances are present in this preparation, that they would have the same threshold value as the hatching factor, so that explanation of this negative result along the above lines is improbable. Clearly, a negative result with the frog heart with a single concentrate possessing hatching properties could justify abandoning the lactone hypothesis. But we do not think that this is justified in the present case. All twelve preparations we have examined which possessed hatching properties, have given colour tests which, on the whole, are reasonably consistent with hatching assay; however only eleven stimulate the hypodynamic frog heart. Either the heart activity is due to the hatching factor, or it is due to some substance accompanying it. If the latter is true, the accompanying

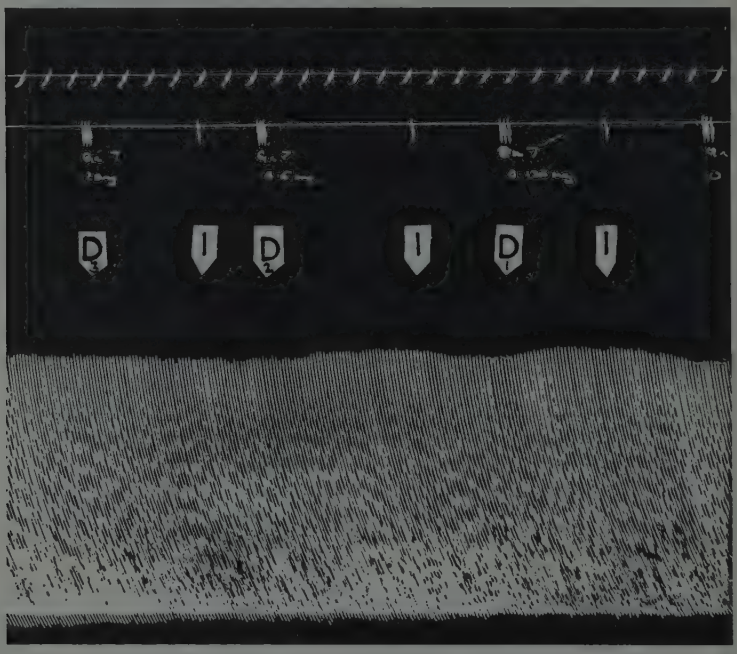

Fig. 4. Isolated frog heart preparation failing to respond to 20 mg./ of preparation G_7/ 100 ml. (D_3). but responds at 0.5 mg./100 ml. (D_2) and 0.125 mg./100 ml. (D_1). Each test solution is followed by low calcium Ringer (!).

substance will, eventually, be left behind as greater purity of the hatching factor is achieved. On this reasoning such a preparation would possess no cardiotonic properties; but, on the other hand, it is reasonable to assume that it would possess very great hatching properties, greater than that of the other preparations which, in this argument, are assumed to be less pure. This is not so in the present case; the preparation which possesses no cardiotonic properties is very far from being the strongest hatching agent. For this reason we feel that it is still for the moment possible to maintain the lactone hypothesis.

It is a pleasure to acknowledge the support of the Nuffield Foundation; our grateful thanks are also due to Dr. G. J. Janzen, of the Proefstation voor Aardappelverwerking, Groningen, for her kindness in supplying us with samples of her concentrates.

ZUSAMMENFASSUNG

Fortschritte im Studium der Physiologie des Schlüpffaktors des Kartoffelnematoden (Heterodera rostochiensis *Woll.*)

Die cardiotonische Aktivität von Substanzen mit Schlüpfreizwirkung beruht weder auf einer Verunreinigung mit Ionen noch auf dem Gehalt an Verbindungen des Inosit-Typs. Die Beeinflussbarkeit des Aktivierungsvorgangs durch Wasserstoffsuperoxyd, sein kolorimetrischer Nachweis, die Empfindlichkeit des Schlüpfprozesses gegenüber der Ionenkonstitution sowie der Einfluss solcher Substanzen, wie Chininsulfat, Dinitrophenol, Acetylcholin und Veratridin auf das Schlüpfen werden verständlich durch die Annahme, dass der Schlüpffaktor eine Affinität zu den Laktonen der Herzglykoside besitzt.

Obwohl der Unterschied in der Empfindlichkeit der Frosch- und Krötenherzen zum Schlüpffaktor nicht so gross ist wie auf Grund der Laktonenhypothese erwartet werden müsste, zeigt sich doch, dass es im Gegensatz zur Theorie HAJDUS (1957) möglich ist, Reaktionen auf das Krötenherz mit einfachen Laktonen, wie γ-Butyrolakton und β-Propriolakton zu erhalten. Von 12 geprüften wirksamen Konzentraten löste ein Stoff, der leidlich übereinstimmende Ergebnisse mit Schlüpf- und kolorimetrischen Versuchen brachte keine Stimulation beim Froschherzen aus. Da dieser Stoff jedoch weit von dem am stärksten wirksamen Reizstoff entfernt ist, lässt sich die Laktonhypothese des Schlüpffaktors noch aufrecht erhalten.

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ÜBER DEN WERT VON SCHWANZFORM UND KÖRPERMAßEN FÜR DIE ARTDIAGNOSE DER NEMATODEN (DARGESTELLT AN DER GATTUNG *HETERODERA* SCHM.)

VON

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In der neueren Literatur, die sich mit der morphologischen Artdiagnose von Nematoden befaßt, finden sich immer häufiger Hinweise auf eine relativ große Veränderlichkeit der Körpermaße und auch der Gestalt einzelner Körperteile der untersuchten Arten. Dies ist besonders dann der Fall, wenn ein größeres Material zur Untersuchung zur Verfügung steht oder wenn verschiedene Herkünfte oder Exemplare von verschiedenen Wirtsarten vergleichsweise untersucht werden konnten. So berichtet schon SEIDENSCHWARZ (1923) von einer Reihe abweichender Schwanzformen bei der heutigen Gattung *Eucephalobus* Steiner und der Art *Dorylaimus carteri* Bastian, die ihm Anlaß gaben, eine Aufgliederung der Arten in Subspecies, Formae und Subformae vorzunehmen. In neueren Untersuchungen weisen TAYLOR & JENKINS (1957) auf die Variationsmöglichkeiten in der Gattung *Pratylenchus* Filipjev hin, TARJAN (1957) unterstreicht für *Ecphyadophora tenuissima* de Man den formverändernden Einfluß verschiedener Wirtspflanzen in verschiedenen Ländern und nimmt das Auftreten geographischer Variationen an. PAETZOLD (1958) konnte für *Rhabditis* (*Rhabditella*) *axei* (Cobbold) Dougherty unter dem Einfluß verschiedener Zuchtmedien erhebliche Längenvariationen und Aberrationen im Bereich des Schwanzes nachweisen. Schließlich sei auf die Untersuchungen von VAN WEERDT (1958), PAESLER (1957a und b) und REUVER (1959) verwiesen, die ebenfalls für die untersuchten Arten deutliche Variationen feststellen konnten.

Beim Arbeiten mit *Heterodera*-Larven bot sich Gelegenheit, eine größere Zahl von Larven II von *Heterodera schachtii* und *H. rostochiensis* auf das Vorkommen von morphologischen Abweichungen zu untersuchen. Die nahezu eine Viertelmillion dazu herangezogener Tiere ließ es zu, ein Bild von der natürlichen Häufigkeit und Art der wichtigsten Abweichungen von der Norm zu gewinnen. Dies schien umso wichtiger, als Untersuchungen über die Häufigkeit des Auftretens solcher Aberrationen noch nicht vorliegen.

Dabei zeigte es sich, daß derartige Abweichungen am häufigsten im Bereich der

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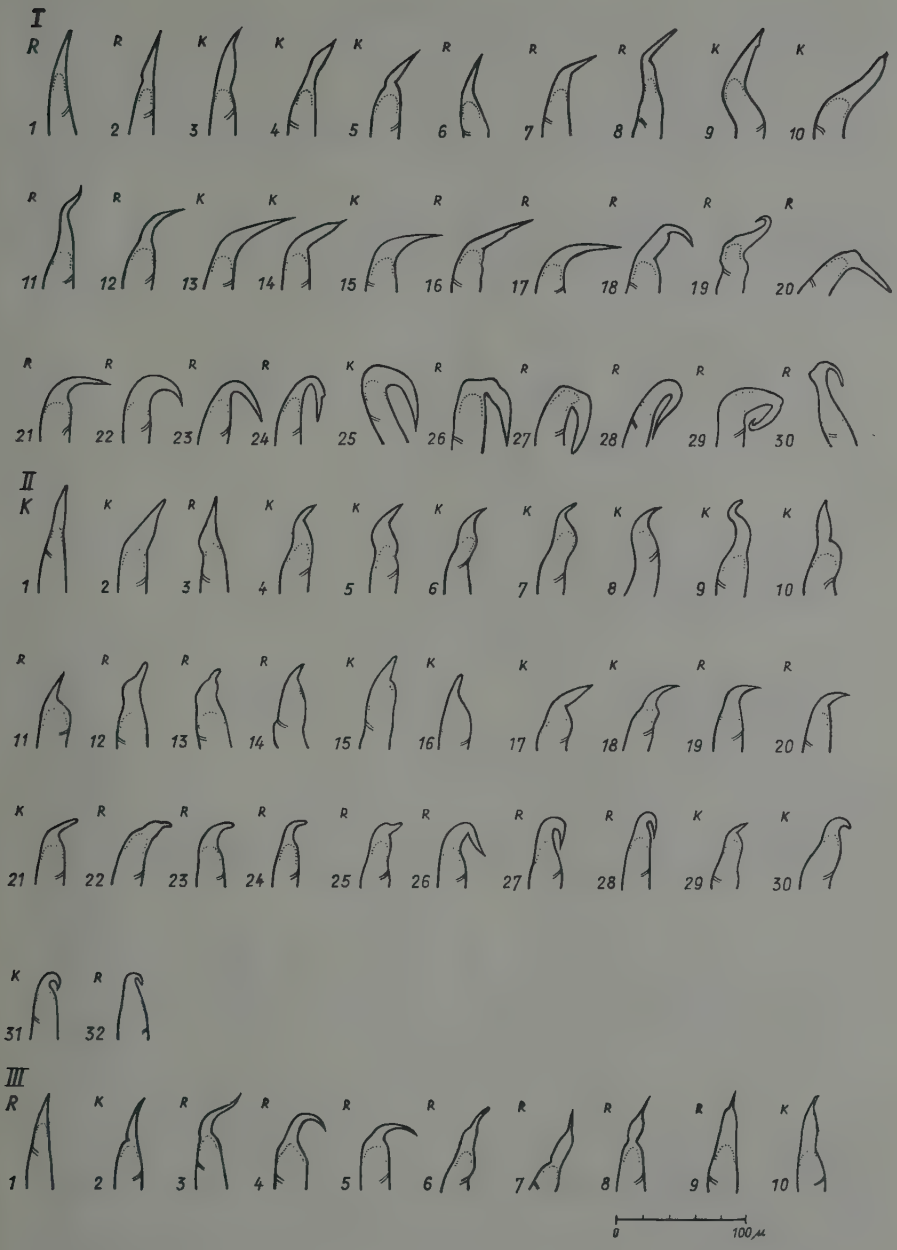

Abb. 1. Schwanzaberrationen bei Rüben- und Kartoffelnematoden-Larven. Nr. 1 jeder Gruppe = normales Schwanzende. Gruppeneinteilung I-VI siehe Text. K = Kartoffelälchen; R = Rübenälchen.

Schwanzspitze erfolgen. Sie treten, wie die Abb. 1 und 2 zeigen, vor allem als Krümmungen (I, II), Spitzenreduktionen (III), postanale Schwanzverdickungen (V) und Abrundung der Schwanzspitze (IV) auf.

Für die hier aufgeworfene Fragestellung ist es von größter Bedeutung, in welchen Häufigkeiten Aberrationen vorkommen. Für 104 492 untersuchte Kar-

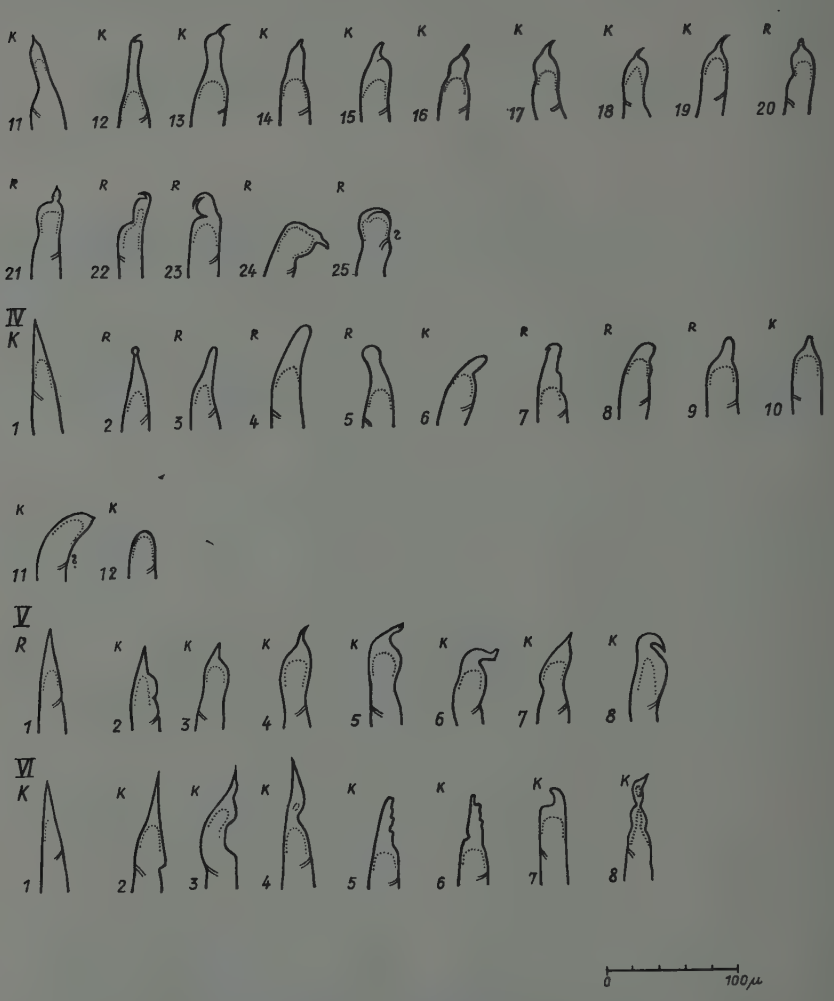

Abb. 2. Schwanzaberrationen bei Rüben- und Kartoffelnematoden-Larven. Legende wie Abb. 1.

toffelälchen fanden sich 84 abnorme Bildungen, was einer Häufigkeitsrate von 1 : 1253,5 entspricht; an 101 809 Rübenälchen traten 83 derartige Abweichungen mit einer Häufigkeit von 1 : 1230,1 auf. Dies Verhältnis anomaler zu normalen

Larven ist also für beide Arten praktisch gleich. Es soll in diesem Zusammenhang nur auf die von OSCHE (1958) erwähnten „Parallelvariationen", denen das „Gesetz der homologen Variationen" von VAVILOV (1922) zugrunde liegt, hingewiesen werden.

Die gefundenen Abweichungen scheinen die Vitalität der Larven nicht wesentlich zu beeinträchtigen: Es wurden für die Gesamtheit der Aberrationen im Schwanzbereich 77,3 % lebender Tiere gefunden.

Auf rund 23 000 untersuchter Tiere entfiel eine keulenförmige Verdickung des Körpers, die zudem mit einer auffallenden Verkürzung des Körpers ein-

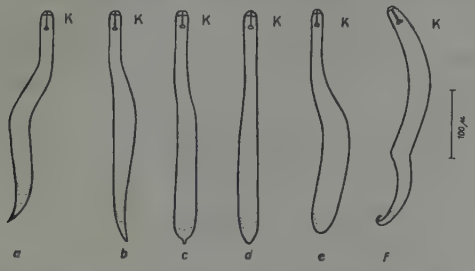

Abb. 3. Beispiele für Körperverdickungen und Verkürzungen bei *Heterodera*-Larven.

herging. Kopfmißbildungen traten nur in einer Häufigkeit von 1 : 69 000 auf. Ca. 7 % der Schwanzanomalien waren mit anderen schweren Mißbildungen des Körpers gekoppelt (Abb. 3).

Zusammenfassend läßt sich feststellen, daß die Schwanzspitze offenbar der labilste Körperabschnitt ist und die Tiere durch solche Bildungsabweichungen kaum beeinträchtigt sind. Gerade die Form des Schwanzes wird aber vom Systematiker zur morphologischen Artdiagnose weitgehend herangezogen. Es scheint deshalb einige Vorsicht geboten, wenn es sich um die Frage der Aufstellung neuer Arten, Unterarten oder Varietäten auf Grund vereinzelt gefundener Tiere mit abweichender Schwanzform handelt.

Über die Ursachen, die zur Bildung der beschriebenen Erscheinungen führen, kann vorläufig noch nichts ausgesagt werden. Es besteht aber der Eindruck, daß Störungen der Lage- und mechanischen Druckverhältnisse während der Embryonalentwicklung zu derartigen Abweichungen von der Normalform führen können.

Das Studium der Aberrationen kann — wie die Arbeiten von OSCHE (1954, 1955, 1958) eindrucksvoll gezeigt haben — über die Beziehungen zur Taxonomie hinaus auch einen Aussagewert für phylogenetische Fragestellungen speziell bei Nematoden gewinnen. Das Variieren morphologischer Strukturen kann bisweilen Ausdruck latenter Entwicklungspotenzen oder atavistisch auftretender alter Strukturen sein und für die Festlegung von Zwillings- und Komplementärarten Hinweise geben.

Wenden wir uns nun dem zweiten wichtigen Merkmal zu, das für die taxono-

mische Artdiagnose der Nematoden von großer Bedeutung ist, nämlich den Körpermaßen, besonders aber der Körperlänge. In einer eingehenden Untersuchung über den Vergleichswert von Längenmaßen zur artlichen Einordnung von *Heterodera*-Larven haben FENWICK & FRANKLIN (1942) auf die Fehlerquellen bei der Meßtechnik aufmerksam gemacht. Ihre Messungen erfolgten vorwiegend an fixiertem Material. Als wichtigen Befund konnten die genannten Autoren sicherstellen, daß die künstlich aus den Zysten freigesetzten Larven in charakteristischer Weise an Länge zunehmen. Damit kommt der Zeit der Messung eine große Bedeutung für die Größe der Längenveränderungen zu. In jüngster Zeit hat MÜLLER (1958) diese Frage wieder aufgegriffen. Sie gibt den Zeitpunkt von 4 Stunden nach dem Freisetzen der Larven aus den Zysten als besonders günstig an und weist darauf hin, das die Mehrzahl der bisher veröffentlichten Maßangaben wegen unzulänglicher Meßtechnik, Fehlens von Angaben über angewandte Präparier- und Meßverfahren und Zahl der vorgenommenen Messungen als unzuverlässig zu bezeichnen sind. Ein Blick auf die großen Unterschiedlichkeiten der bisher veröffentlichten Larvenmaße in der Gattung *Heterodera* Schm. bestätigt diesen Hinweis.

Daß die Veränderung der Längenmaße aber wesentlich auch von anderen Faktoren als dem schon von FENWICK & FRANKLIN (1942) erkannten Zeitpunkt der Messung abhängig ist, zeigen die folgenden Untersuchungen.

Im Rahmen von Beobachtungen über den Einfluß osmotisch wirksamer Medien auf *Heterodera*-Larven war die oben erwähnte Längenzunahme der Larven in Leitungswasser ebenfalls festgestellt und näher untersucht worden. Dabei ergab sich für eine Meßdauer von 5 Tagen ein Längenzuwachs wenigstens bis zum 2. Tag. Der prozentual größte Zuwachs liegt innerhalb der ersten 6 Stunden nach Öffnen des Zysten. Dabei war dafür Sorge getragen worden, daß die Ausschlämmung der Zysten schnellstens erfolgte und die Messungen unmittelbar danach beginnen konnten. Die zystenhaltige Erde war relativ trocken gewesen (Abb. 4).

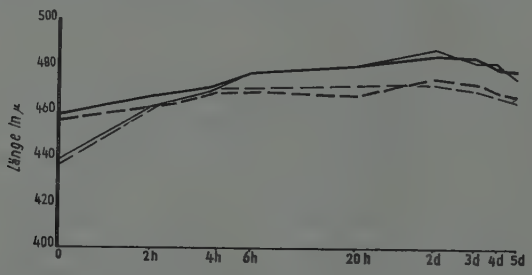

Abb. 4. Längenveränderungen an *Heterodera*-Larven in Leitungswasser nach Öffnen relativ trockener (dünne Kurven) und vorgeweichter Zysten (dicke Kurven). Halblogarithmisch. —— = Kartoffelnematoden; — — — = Rübennematoden.

In einem Parallelversuch ist das Zystenmaterial 10 Tage auf feuchtem Fließpapier vorgeweicht worden. Dadurch liegt die Ausgangsgröße der Larven um ca.

20 μ höher als im ersten Versuch, und der steile Anstieg der Längenwerte in den ersten 2 Stunden ist dadurch erheblich gemildert. Nach etwa 4 Stunden zeigen die Kurvenzüge beider Versuche praktisch gleichen Verlauf. Der erreichte Höchstwert für beide Nematodenarten liegt auch hier am 2. Tage. Damit ist erwiesen, daß neben den von FENWICK & FRANKLIN (1942) und MÜLLER (1958) aufgezeigten Faktoren auch die Art der Vorbehandlung des Zystenmaterials in bezug auf ihre Feuchthaltung von großer Bedeutung für die Längenveränderungen ist. Der Einfluß vorheriger Trockenhaltung führt vor allem in den ersten 48 Stunden nach Öffnen der Zysten zu erheblichen Unterschieden, die bis zu 20 μ betragen können. Um vergleichbare Werte für die Längenangaben zu erzielen, ist es deshalb unbedingt erforderlich, den Zysten eine einheitliche Behandlung angedeihen zu lassen, wobei am besten mit ausreichend feuchtigkeitsgesättigten Zysten zu arbeiten ist, weil dann die Quellungserscheinungen am geringsten sind. Die Zeitdifferenz von 4 Stunden, die MÜLLER (1958) als zweckmäßig für den Beginn der Messungen erachtet, ist dann auch auf Grund unserer Befunde zu empfehlen.

Mit der Beachtung des Feuchtigkeitszustandes des Zystenmaterials allein sind die möglichen Fehlerquellen für die Meßergebnisse jedoch noch nicht erschöpft. Im Verlauf der Messungen war der Eindruck entstanden, daß tote Älchen sich stärker verändern als lebende. Es war zur Klärung dieser Frage also notwendig, bei den Messungen lebende und tote Tiere zu trennen. Eine Abtötung der Larven durch Erwärmung zeigte aber die bereits von FENWICK & FRANKLIN (1951) festgestellte Schrumpfung, so daß diese Art der gewaltsamen Abtötung ausscheiden mußte. Diese Abtötungsmethode ist deshalb nur mit der allergrößten Vorsicht anzuwenden, weil ständig die Gefahr einer Überhitzung bei der Abtötung über der Flamme besteht. Auch für andere Nematodenarten wird diese Abtötungsart herangezogen, und auch in diesen Fällen ist mit einer unkontrollierten Beeinflussung der Längenmaße zu rechnen.

Auf Grund dieser Erfahrungen wurde mit natürlichem Zysteninhalt gearbeitet, der immer einen unterschiedlich großen Prozentsatz abgestorbener Larven enthält. Mit Hilfe des Vitalfarbstoffes Chrysoidin wurde der Inhalt angefärbt, nachdem vorher die Länge der Larven ohne Rücksicht auf Lebende und Tote festgestellt worden war. Außerdem sind Zysten benutzt worden, die vorher ausreichend lange feucht gehalten waren. Bei der dann folgenden getrennten Messung lebender und toter Tiere wurden nur solche herangezogen, die einwandfrei die Vital- bzw. Letalkriterien zeigten. Für die Toten wurde außerdem darauf geachtet, daß die gemessenen Tiere frei von Verletzungen waren und noch keine fortgeschrittenen postmortalen Veränderungen zeigten. Allgemein wurden nur natürlich gestreckte Larven vermessen.

Der Vergleich der Längenmaße lebender und toter Tiere zeigt nun tatsächlich den vermuteten Unterschied (Abb. 5). Zunächst war wieder der anfängliche Anstieg der Längenwerte zu konstatieren. Vom Zeitpunkt der wirksam gewordenen Anfärbung divergieren die Längenkurven beider *Heterodera*-Arten für lebende und tote Larven erheblich, wobei die Werte für die Toten noch stark

ansteigen, während für die Lebenden die Werte nahezu konstant bleiben oder bei längerer Versuchsdauer um ein Weniges absinken. Kartoffel- und Rübennematoden zeigen dabei die gleiche Tendenz. Auch die absoluten Längenwerte, die von den Toten beider Spezies erreicht werden, liegen in einem Niveau und 30 bis 40 μ höher als die der lebenden Larven. Für diese sind die gemessenen Werte für die Kartoffelnematoden etwas höher, was sich mit den früheren Befunden durchaus deckt. Berechnet man den Längendurchschnitt, so ergibt sich ein Kurvenverlauf, der etwa dem in Abb. 4 entspricht.

Damit dürfte gleichzeitig der Beweis erbracht sein, daß bei der Prüfung von künstlich aus den Zysten freigelegten Larven die Längenwerte in starkem Maße von dem Anteil der toten Tiere in einem solchem Gemisch abhängig sind. Eine Vergrößerung dieses Anteils muß zwangsläufig ein Anwachsen der Längenmaße

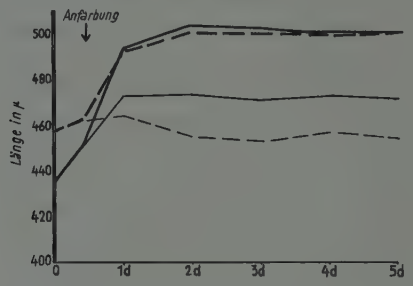

Abb. 5. Längenveränderungen künstlich freigelegter Heterodera-Larven bei Trennung in lebende und tote Tiere. —— = lebende; — = tote Kartoffelnematoden; — — = lebende; — — = tote Rübennematoden.

mit sich bringen. Da die Quellung der toten Larven über etwa 2 Tage anhält, ist auch hier der Zeitpunkt der Messung nach Freisetzen der Larven von großer Bedeutung. Die auffallend hohen Werte, die MÜLLER (1958) ihren Messungen entnommen hat, können ihre Ursache in einem relativ großen Anteil toter Larven haben.

Der Befund, daß auch lebende Larven zu Längenzunahmen neigen, führte zu der Überlegung, daß die Längenzunahme unter der Einwirkung osmotisch wirksamer Medien nicht eintreten dürfte. Entsprechende Versuche unter Benutzung von 0,1-, 0,2-, 0,3-, 0,4-, und 0,5- molaren Kochsalzlösungen ließen aber für den Kartoffelnematoden in jedem Falle einen deutlichen Längenzuwachs erkennen. Für den Rübennematoden war er weniger stark ausgeprägt. Durch die 24-stündige Vorbehandlung der benutzten Zysten in den betreffenden Lösungen waren die Ausgangslängen der Larven in Abhängigkeit von der molaren Konzentration deutlich abgestuft kürzer. Dann setzt jedoch in allen Fällen die Längenzunahme ein.

Um weiter zu prüfen, in wieweit schon innerhalb der Zysten eine Angleichung an das gebotene osmotische Milieu einsetzen kann, wurden die Zysten verschieden lange Zeiten mit den Lösungen vorbehandelt, in denen dann auch die Larven frei-

gesetzt und gemessen wurden. Es ist dabei zu betonen, daß sich das osmotische Milieu während der Vorbehandlung und der Freisetzung der Larven nicht änderte. Aber auch hier treten nach Freiwerden der Larven in allen Konzentrationen Längenzunahmen von 20 bis 25 μ auf, wenn auch eine graduelle Abstufung mit der Höhe der Konzentration gegenüber der Kontrolle vorhanden ist.

Auf Grund des bisherigen Bildes kann geschlossen werden, daß mit der Freisetzung der Larven stets eine Wasseraufnahme und damit eine Längenzunahme einhergeht. Diese können u.U. auch entgegen osmotischen Kräften erfolgen. Hingegen besteht der Eindruck, daß die noch von der Zysten- und Eihülle umschlossenen Larven noch nicht voll in der Lage sind, ihren Organismus osmotisch richtig einzuregulieren. Erst nach dem Freiwerden der Larven scheint diese Fähigkeit wirksam zu werden. Zur Einregulierung ihres endogenen Wasserhaushaltes kommt

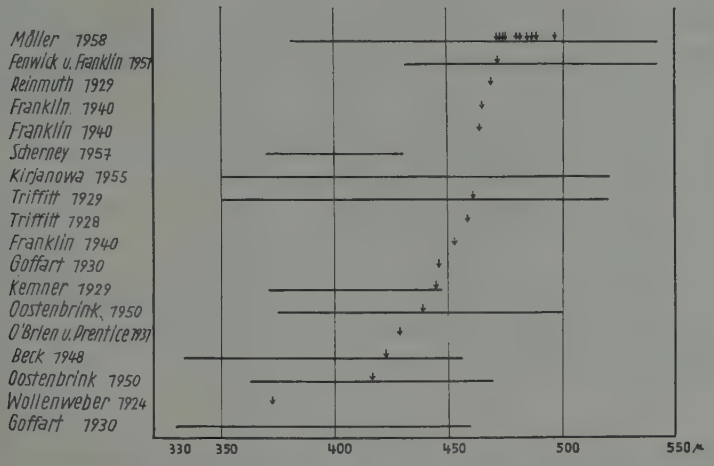

Abb. 6. Durchschnittswerte (senkrechte Pfeile) und Extremwerte (waagerechte Linien) für die Larvenlänge des Kartoffelnematoden nach Angaben verschiedener Autoren.

es dabei zu den beobachteten Längenzunahmen unmittelbar nach dem Freiwerden der Larven. Unter diesem Gesichtspunkt erscheint auch die Feststellung CHITWOODS (1952) in neuem Licht, daß nämlich die Larven unmittelbar nach dem Schlüpfen eine größere Permeabilität und Giftempfindlichkeit zeigen. Natürlich geschlüpfte Larven dürften als Ausdruck ihrer Schlüpfbereitschaft die Fähigkeit zur Regulation ihres Wasser- und osmotischen Gleichgewichts schon in weit ausgereiftem Grade entwickelt haben, was bei künstlich freigelegten Larven noch nicht der Fall ist. Dies macht die deutlichen Längenveränderungen bei diesen verständlich. Auch eigene Befunde über die größere Aufnahme von Vitalfarbstoffen unmittelbar nach künstlichem Freisetzen der Larven sprechen für eine größere Permeabilität der Kutikula zu diesem Zeitpunkt. Der teilweise nach etwa 2 Tagen einsetzende Rückgang der Längenwerte kann als Ausdruck der erfolgten Einregulierung des Wasserhaushalt betrachtet werden.

Im Hinblick auf die wichtige Eigenschaft der Längenmaße für die Artdiagnose müssen auf Grund der Befunde folgende Punkte zusätzlich zu den bisher bekannten Fakten unbedingte Beachtung finden:

1. Der Zeitpunkt der Messung nach der Eröffnung der Zysten ist zu beachten. Er soll etwa bei 4 Stunden nach Freilegung der Larven liegen.
2. Die Vorgeschichte des Zystenmaterials in Hinblick auf die Feuchthaltung und auf die osmotischen Bedingungen ist für die Veränderlichkeit der Längenmaße von großem Einfluß. Es sollten nur gut durchfeuchtete Zysten benutzt werden.
3. Der Anteil der toten Larven, die sich stärker verändern als die lebenden Tiere, wirkt unmittelbar auf die Veränderlichkeit der Längenwerte ein. Es sollten daher nur lebende Tiere zur Messung herangezogen werden.
4. Weil das osmotische Regulationsvermögen offenbar bei künstlich freigelegten Larven noch nicht voll entwickelt ist, empfiehlt es sich, daß nach Möglichkeit selbstständig geschlüpfte Larven zur Feststellung der Maße benutzt werden. Damit entfallen die unter 1-3 genannten Vorsichtsmaßnahmen weitgehend.

Die Nichtbeachtung der einzelnen Punkte wird als der Hauptgrund für die stark divergierenden Längenangaben bei zystenbildenden Nematoden angesehen, die damit erheblich an Aussage- und Vergleichswert verlieren (Abb. 6).

Die Forderung nach der kritischen Prüfung des Wertes von Schwanzformen und Körperlängen-Angaben bei der Determination von Nematoden dürfte auf Grund der dargelegten Befunde auch über die zystenbildenden Nematoden hinaus berechtigt sein. Gerade auch freilebende Nematoden dürften auf Änderungen des osmotischen Mediums bei der Messung sehr empfindlich reagieren, und auch bei ihnen ist mit Längenveränderungen nach Hitzeabtötung zu rechnen. Die Prüfung ihres Verhaltens unter den entsprechenden Bedingungen ist deshalb vorgesehen.

SUMMARY

On the value of tail form and body measurements in the diagnosis of nematode species (illustrated by the genus Heterodera *Schm.)*

More than 200,000 larvae of *Heterodera schachtii* Schmidt and *H. rostochiensis* Wollenweber were examined for morphological aberrations. Abnormalities in the form of the tail were most common, occurring in about one in 1,250 larvae and suggesting that the tail is morphologically the most labile region of the body. These modifications, if they possessed any function, did not appear to affect the organism. Other anomalies are described and their significance as diagnostic characteristics considered. Variations in measurements which supplement those given in the literature are described. They arise as follows: (1) through measurement at different times after emergence from cysts, (2) through the different conditions of moisture and osmotic pressure to which larvae and cysts have been subjected, (3) through the different proportions of dead and living larvae in the sample under examination and (4) through the wide variations in the power of osmotic regulation between larvae. Lack of consideration of these and other known causes of variation appear responsible for wide variations in measurements of *Heterodera* larvae.

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DISKUSSION

P. A. A. Loof: In diesem Zusammenhange möchte ich hinweisen auf den Einfluss des Fixierens und des Einschliessens auf die Masse der Nematoden. Eigene Untersuchungen haben herausgestellt, dass beim üblichen F. A. A.-Glyzerin-Verfahren die Länge der Aelchen durchschnittlich um etwa 15% abnimmt, in einzelnen Fällen sogar bis 25%. Die Indizes V, a, b und c ändern sich dagegen kaum. Nur bei zusammengeklappten Tieren ist — bei sehr geringer Längenabnahme — der Index a stark erhöht. Es empfiehlt sich, bei Neubeschreibungen anzugeben, ob die Masse sich auf frische oder präparierte Stücke beziehen, und womöglich Masse beider zu geben.

L. Kämpfe: Der Hinweis stellt eine wichtige Ergänzung dar, zumal im Vortrag nicht näher auf den Einfluss des Fixierens eingegangen werden konnte. Er unterstreicht die Notwendigkeit, die Massangaben so kritisch wie möglich zu bewerten.

Nematologica (Suppl. II, 1960): 123-126. — E. J. Brill, Leiden.

OBSERVATIONS ON THE CYTOLOGY OF THE POTATO-ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* WOLLENWEBER

BY

J. COTTEN

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A study was made of the meiotic divisions of oogenesis, and also of the early cleavage divisions of the egg of the potato-root eelworm *Heterodera rostochiensis* Wollenweber. During the first division of meiosis, the chromosomes are about 1 μ in size; during the cleavage divisions they are very much smaller, and at this stage the individual chromosomes are not resolvable with the light microscope.

White females of the potato-root eelworm were fixed either in Carnoy's fluid or in 1 : 3 propionic alcohol and stained with 1 % orcein in 45 % propionic acid using a normal squash technique (MULVEY 1955). For determination of chromosome number, the coverslip was lifted off, the body wall of the female removed, and the preparation re-squashed using considerable pressure. In this way is was usually possible to bring the chromosomes into one plane.

At early first meiotic prophase, the chromosomes stain faintly but seem to be paired. At late first meiotic prophase the chromosomes appear as deeply staining bodies (Fig. 1). It is difficult to identify the individual members of a pair at this stage. Most eggs in late first meiotic prophase seem to contain a sperm nucleus, but this may not always be the case. Sometimes during preparation of the squash the sperm may become obscured; this could explain its absence in some first meiotic prophase eggs.

By first meiotic metaphase, all eggs normally contain a sperm nucleus. At this stage, the chromosomes are arranged in pairs across the equatorial plate of the spindle, at right angles to the long axis of the egg (Fig. 2). As the chromosomes move apart, the entire spindle moves through 90°, so that by first meiotic telophase, one pole of the spindle is lying against the egg wall (Fig. 3). As first meiotic metaphase is the most frequently occurring stage, this would seem to be the longest stage in the division.

The egg chromosomes now go through a second division. Fig. 4 shows second meiotic prophase and sperm nucleus; the first polar body is out of focus on this photograph. Fig. 5 shows second meiotic metaphase and the first polar body. Fig. 6 shows a polar view of second meiotic anaphase, and the first polar body.

As a result of these two divisions, the egg now contains two polar bodies, the

egg chromosomes which are now assumed to be in the haploid condition, and a sperm nucleus. Due to the extremely small size of the chromosomes, it has not been possible to determine the somatic chromosome number. It therefore can only be assumed that as a result of the two divisions, the egg chromosomes are left in the haploid condition.

Despite examination of some 500 females, fusion between egg chromosomes and sperm nucleus has never been observed. This is not proof that a fusion does not occur: failure to observe this stage in the process could be due to the unsuitability of the fixing and staining technique.

Chromosome number was determined mainly from preparations showing first meiotic metaphase, but also from late prophase and early anaphase figures. From these determinations it is apparent that the chromosome number is not constant. Metaphase figures with nine, ten and eleven pairs of chromosomes have been observed (Figs. 9, 10, 11). Occasionally univalents i.e., unpaired chromosomes, have also been observed (Fig. 12). In most preparations, only a single egg gives a suitable figure, so that most of the following results are based on only one determination per female. Occasionally more than one egg may be suitable, but the maximum number of determinations per female was only three. In the few cases where it was possible to make more than one determination on a single female, the number was constant for the different eggs.

Fig. 13 shows the chromosome number of eggs of females obtained from the following potato varieties: Majestic in 1958, Redskin in 1958 and Sharpe's Express in 1959. There is a slight indication that females with eggs having twenty chromosomes occurred more frequently on Sharpe's Express plants than on Redskin, and on Redskin plants more frequently than on Majestic. However there are not sufficient data available to reach any conclusions on this point, and in any case, these differences if they are real, may not be due to differences between the potato varieties, but to variations in experimental conditions.

Riley & Chapman (1957) have already reported that the diploid chromosome number of the potato-root eelworm is eighteen, but without information as to the number of animals which they examined, it is difficult to know if the variation I find is due to differences in population.

This variation in chromosome number now raises problems of fertilization. Ellenby (1957), Fassuliotis (1957) and Williams (1957) have independently shown that parthenogenesis did not occur in the potato-root eelworm populations they tested, and that the sperm must play some part in the reproductive process. I have not as yet been able to determine the chromosome number of the male; however if all the chromosome types are to be maintained, the chromosome number of the male must also be variable. Now if a male is able to mate with any female irrespective of its chromosome number, a high proportion of odd chromosome numbers should arise in the progeny of such matings. Figs. 13a, 13b and 13c show that this is not the case; univalents do occur, but they are infrequent.

Although a fusion stage between sperm and egg nucleus was never found, a

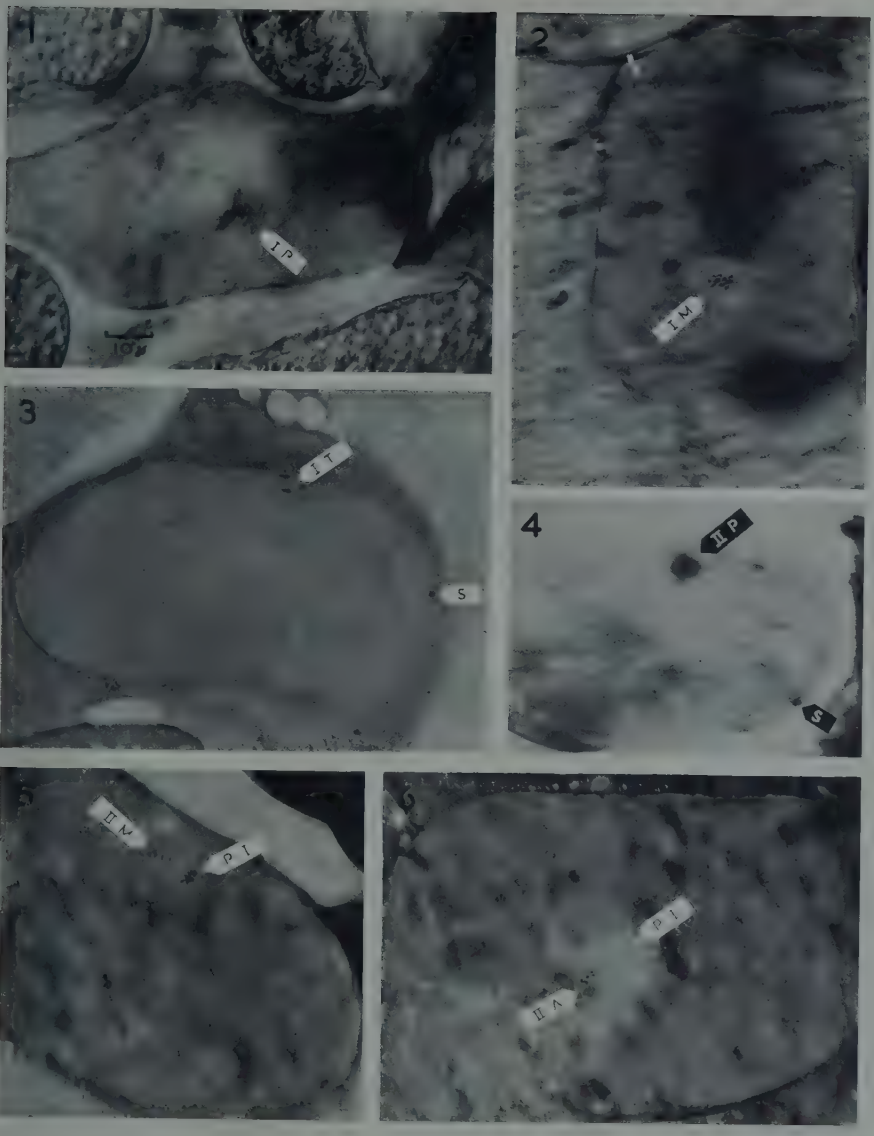

Fig. 1. Egg in first meiotic prophase. IP — prophase chromosomes.
Fig. 2. Egg in first meiotic metaphase. IM — metaphase chromosomes.
Fig. 3. Egg in first meiotic telophase. IT — telophase chromosomes. S — sperm.
Fig. 4. Egg in second meiotic prophase. IIP — prophase chromosomes. S — sperm.
Fig. 5. Egg in second meiotic metaphase. IIM — metaphase chromosomes. PI — first polar body.
Fig. 6. Egg in second meiotic anaphase. IIA — anaphase chromosomes PI — first polar body
Figs. 1-6 are all to the same scale.

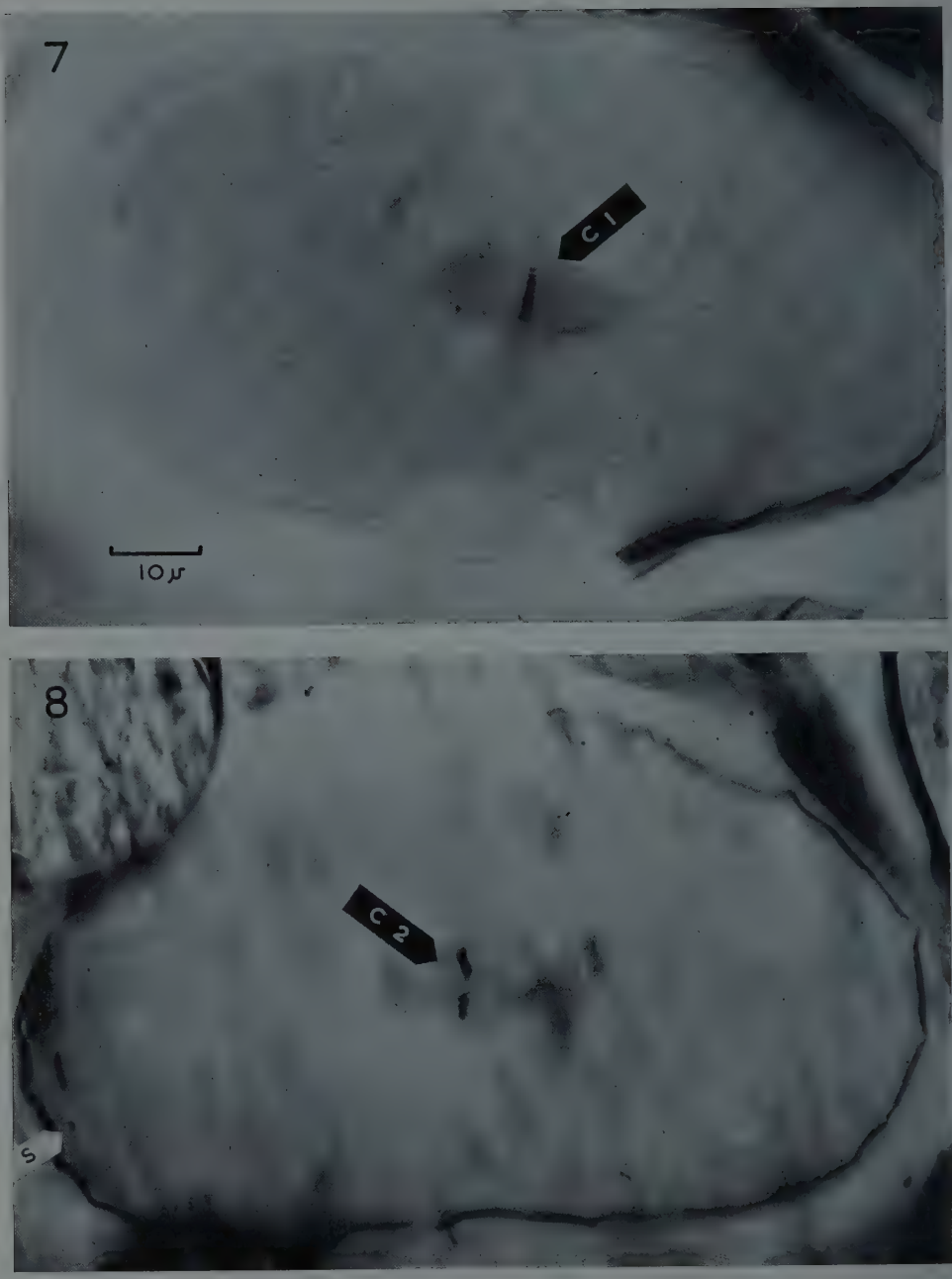

Fig. 7. Egg in metaphase of the first cleavage division. CI — metaphase chromosomes arranged in one group.

Fig. 8. Egg in metaphase of the first cleavage division. C2 — metaphase chromosomes arranged in two groups. S — remains of sperm nucleus.

Fig. 7 & 8 are to the same scale.

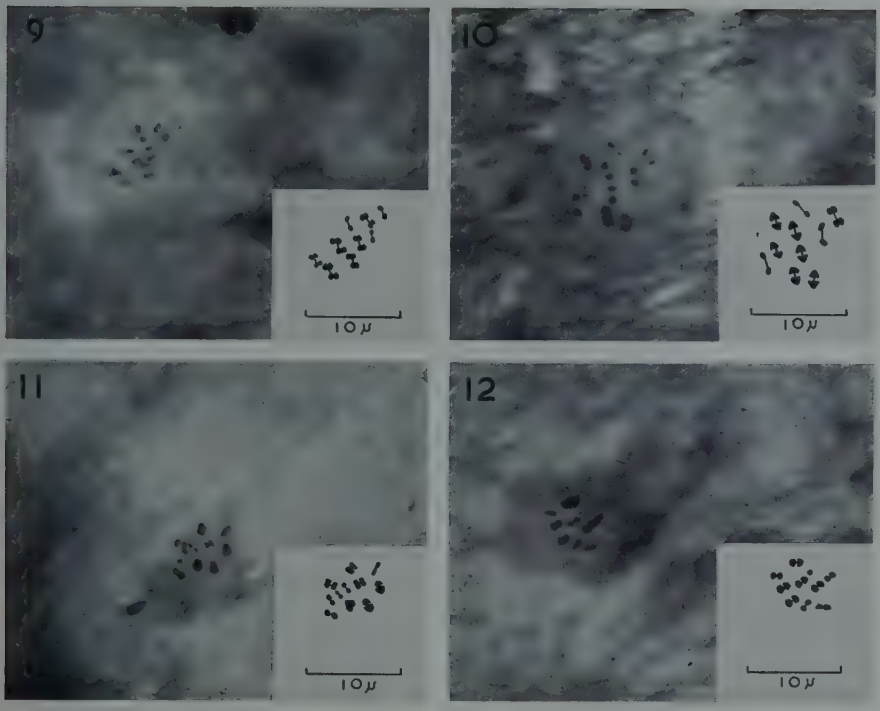

Fig. 9. First meiotic metaphase figure with 9 pairs of chromosomes.
Fig. 10. First meiotic metaphase figure with 10 pairs of chromosomes.
Fig. 11. First meiotic metaphase figure with 11 pairs of chromosomes.
Fig. 12. First meiotic metaphase figure with 9 pairs of chromosomes and one univalent.

J. COTTEN: *Cytology of the potato root eelworm.*

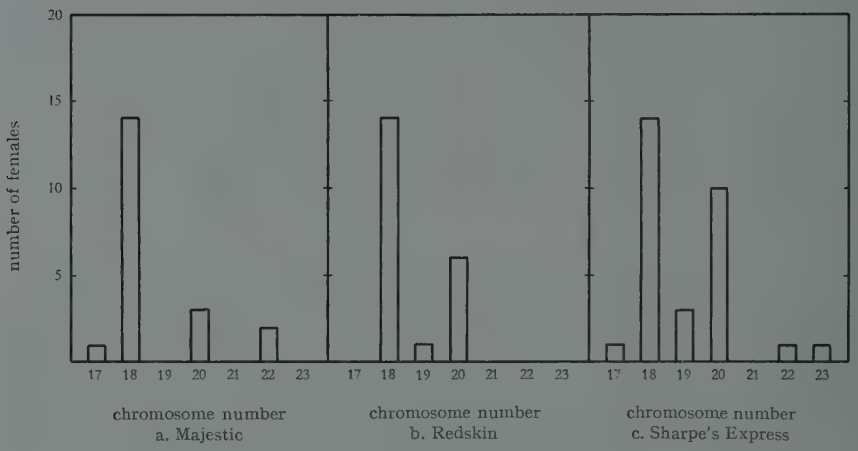

Fig. 13. Chromosome number of eggs of females from three potato varieties.

number of first cleavage divisions were observed. These were of two types. Fig. 7 shows the first type where at metaphase, the chromosomes are arranged in one line across the equator of the spindle. Fig. 8 shows the second type where at metaphase the chromosomes are arranged in two separate groups. This latter type of cleavage division occurs in the brine shrimp *Artemia salina* (BRAUER 1894), which has a pseudogamous mode of reproduction, that is the sperm initiates the division and then degenerates, no fusion occurring between the male and female nuclei. The remains of what appears to be a sperm nucleus can be seen on Fig. 8. 'Double type' cleavage divisions were found on a number of other occasions but no sperm nuclei were observed.

There is some evidence that 'single type' cleavage divisions are associated with females having eggs with eighteen chromosomes, and 'double type' cleavage divisions with females having eggs with twenty chromosomes; but the material is not extensive enough for this to be certain.

Eggs in the second division of meiosis were found in females with eighteen chromosome eggs, and also in females with twenty chromosome eggs. If the latter do have a pseudogamous mode of reproduction, then there must also be some means of restoring the diploid chromosome number. In *Artemia salina* (BRAUER 1894), this is brought about by the fusion of the egg chromosomes and the second polar body. This was never observed in the potato-root eelworm.

Although more than 500 females have been examined, many of the foregoing results are of a preliminary nature, and much more work will have to be done before meiosis and the fertilization process of the potato-root eelworm are clear.

The work is supported by a grant from the Agricultural Research Council, for which I would like to express my thanks.

ZUSAMMENFASSUNG

Beobachtungen über die Zytologie des Kartoffelnematoden

Das befruchtete Ei des Kartoffelnematoden macht zwei Teilungen in der Meiose durch. Die Chromosomenzahl wurde hauptsächlich bei der ersten meiotischen Metaphase bestimmt. Auf diesem Stadium sind die Chromosomen etwa 1 μ gross. Die Chromosomenzahl ist nicht konstant. Wo es aber möglich war, die Chromosomenzahl bei mehr als 1 Ei je Weibchen zu ermitteln, war sie konstant. Tiere wurden gefunden, deren Eier 18, 20 und 22 Chromosomen (diploid) hatten; ungerade Zahlen waren selten. Bei einer Untersuchung von mehr als 500 Weibchen wurde niemals Verschmelzung von Spermakern und Eikern beobachtet. Zwei Typen der ersten Teilung wurden beobachtet, ein normaler „single" Typ und ein „double" Typ. Bei anderen Organismen ist der letztere Typ zuweilen mit Pseudogamie verbunden.

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DISCUSSION

P. A. A. LOOF: From the proportions shown it would seem as if 18 were the normal chromosome number, as in other *Heterodera* species. Did Mr. Cotten ever observe any irregularity in chromosome arrangement in eggs with 2n = 20 or 22?

Also I would like to ask whether eggs of the aggressive biotype were examined, and if so, whether there was any correlation between chromosome number and biotype?

J. COTTEN: At the moment, it would seem that the basic chromosome number of the genus is 9. Chromosome arrangement is only regular at metaphase. Metaphase plates with 10 pairs of chromosomes are as regular as metaphase plates with 9 pairs. I have only three reliable observations of figures with 11 pairs of chromosomes; two of these are prophase figures and cannot be judged in this respect. The third is a regular metaphase. My observations have all been on one population. I have no knowledge of the aggressiveness of this population towards any resistant potato material.

Nematologica (Suppl. II, 1960): 127-140. — E. J. Brill, Leiden

ÜBER DIE SCHLÜPFAKTIVE BZW. SCHLÜPFHEMMENDE WIRKUNG DER WURZELDIFFUSATE VERSCHIEDENER PFLANZEN AUF DEN KARTOFFELNEMATODEN, *HETERODERA ROSTOCHIENSIS* WOLL.

2. MITT. ÜBER *HETERODERA*-ARTEN

VON

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Seit den Untersuchungen von BAUNACKE (1923) am Rübennematoden (*Heterodera schachtii* Schmidt) und von TRIFFITT (1930) sowie O'BRIEN & PRENTICE (1930) am Kartoffelnematoden (*Heterodera rostochiensis* Woll.) ist bekannt, daß das Schlüpfen der in den Eiern ruhenden Larven durch bestimmte, von den Wurzeln der Wirtspflanzen ausgehende Reize aktiviert wird. Für den Kartoffelnematoden geht diese Aktivierung auf Schlüpfstoffe zurück, die im Wurzeldiffusat (Wurzelablaufwasser) einiger Solanaceen, insbesondere der Kartoffel und der Tomate, vorhanden sind (vgl. NOLTE, 1958).

Über Untersuchungen zur Isolierung sowie zur Aufklärung der chemischen Konstitution und Wirkungsweise dieses „hatching factor" wurde in der Literatur mehrfach berichtet.

So ist auf Grund der Arbeiten von Todd und Mitarb. (CALAM, RAISTRICK & TODD, 1949; CALAM, TODD & WARING, 1949; MARRIAN, RUSSELL, TODD & WARING, 1949; MARRIAN, RUSSELL & TODD, 1949; RUSSELL, TODD & WARING, 1949; CARROLL, HEYES, JOHNSON & TODD, 1958), JOHNSON (1952) sowie WIDDOWSON & WILTSHIRE (1958) bekannt, daß dem aus Tomaten-Wurzeldiffusaten isolierten und als „Eclepsäure" bezeichneten Schlüpfstoff voraussichtlich die Summenformel $C_{18}H_{24}O_8$ bzw. nach späteren Untersuchungen die Formel $C_{19}H_{20}O_8$ zukommt [1]). Bei der Eclepsäure handelt es sich nach bisherigen Befunden vermutlich um ein γ-Lacton mit einer zusätzlichen Carboxylgruppe, 1-2 freien Hydroxylgruppen und einer Doppelbindung.

Im Gegensatz zu diesen Ergebnissen gewannen kürzlich HARTWELL, DAHLSTROM & NEAL (1959) aus Tomaten-Wurzeldiffusaten ein kristallines, jedoch im Vergleich zur Eclepsäure offensichtlich weniger aktives Präparat, dem möglicherweise die Elementarzusammensetzung $C_{13}H_{12}O_3$ zukommt. Eine freie Carboxyl-

[1]) Die in Wurzeldiffusaten von *Solanum tuberosum* und *S. nigrum* vorkommenden Schlüpfstoffe sind mit der „Eclepsäure" identisch oder chemisch sehr nahe verwandt.

gruppe konnte in dieser Substanz nicht nachgewiesen werden. Dieser Befund stützt unsere Vermutung, daß der Schlüpfvorgang durch mehrere, in ihrer Wirkungsweise und -stärke sehr differierende Naturstoffe aktivierbar ist.

Das Aktivitätsmaximum der bisher wirksamsten Präparate liegt bei 0,1 mg/L (WIDDOWSON & WILTSHIRE, 1958). Nach ELLENBY & GILBERT (1958) kommt dem „hatching factor" eine herzaktive Wirkung zu, die wie bei anderen herzaktiven Lactonen durch H_2O_2 aktivierbar ist.

Die Wurzeldiffusate (WD) einer Reihe weiterer Solanaceen besitzen diese schlüpfaktivierende Wirkung nicht oder in nur geringem Maße (Tab. I). Zu

TABELLE I

Schlüpfwirkung der Wurzeldiffusate verschiedener Solanaceen

	Relative Schlüpfwirkung der Wurzeldiffusate (WD) in %
Solanum tuberosum L., „Aquila"	100
Solanum abutiloides Bitter et Lillo	35
Solanum atropurpureum Schrank	3
Solanum aviculare Forst.	1-67*)
Solanum auriculatum Ait.	3
Solanum capsicastrum Link	2
Solanum melongena L.	13
Solanum miniatum Bernh.	2
Solanum robustum H. Wendl.	< 1
Atropa bella-donna L.	1-2
Capsicum annuum L., „Süßer"	≈ 1
Cyphomandra betacea Sendtn.	2-5
Datura metel L.	< 1
Fabiana imbricata Ruiz et Pav.	1
Hyoscyamus niger L.	< 1
Lycopersicon hirsutum H. B. et K.	23**)
Lycopersicon mexicanum	134**)
Lycopersicon peruvianum Mill.	39**)
Lycopersicon pimpinellifolium Mill.	70**)
Nicotiana tabacum L., „Virgin Gold A"	≈ 1
Physalis philadelphica Lam.	< 1
Schizanthus retusus Hook.	1-2
Withania somnifera (L.) Dun.	1-11

*) WD von Pflanzen verschiedenen Alters.

**) Die WD-Aktivität wechselt stark mit dem Alter der Pflanzen. Die hier angeführten Werte beziehen sich auf 8 Wochen alte Pflanzen.

diesen gehören u.a. auch solche „Wirtspflanzen", deren Wurzeln nach Infektion mit aktiven Larven eine zwar zahlenmäßig unterschiedliche, jedoch volle Entwicklung des Nematoden zulassen (z.B. *Solanum aviculare, S. melongena,*

S. miniatum, Atropa bella-donna, Cyphomandra betacea, Hyoscyamus niger). Allerdings sei darauf hingewiesen, daß die Schlüpfwirkung der überprüften Wurzelablaufwässer in Abhängigkeit vom Alter und Entwicklungszustand der Pflanzen stärkeren Schwankungen unterworfen sein kann, wie es ähnlich bereits bei der Kartoffel bekannt ist (WIDDOWSON, 1958).

Zur Klärung der Frage, ob die Inaktivität der in Tabelle I aufgeführten Wurzeldiffusate auf das Fehlen von Schlüpfstoffen oder auf vorhandene Hemmstoffe zurückzuführen ist, wurden von uns Mischdiffusate getestet (SCHREIBER & SEMBDNER, 1959).

METHODIK

Die Durchführung der Schlüpfversuche erfolgte unter weitestmöglicher Berücksichtigung und in Anlehnung an die allgemein üblichen Verfahren (FENWICK, 1949, 1952; FENWICK & WIDDOWSON, 1958; KRADEL, 1959; STELTER, 1958). Die für unsere von Frühjahr bis Sommer angesetzten Schlüpfteste erforderlichen Zysten wurden, um gleichaltriges Material zu erhalten, im Vorjahr an Kartoffelpflanzen der Sorte „Aquila" in Blumentöpfen angezogen. Zur Infektion benutzten wir in Beutel aus Perlongaze eingebundene Zysten einer natürlichen Population. Nach Ausschlämmung (Fenwick-Kanne) und Reinigung wurde das Zystenmaterial größenmäßig sortiert und vor Versuchsbeginn zur Beschleunigung des Schlüpfvorganges 3 Wochen lang in täglich gewechseltem Wasser vorgequollen. Aufbewahrung und Vorbehandlung der Zysten sowie Versuchsdurchführung erfolgte bei 22-24° C in temperaturkonstanten Kammern. Für jeden Test verwendeten wir 100 bei schwacher Vergrößerung einzeln ausgelesene Zysten, die zu je 10 Stück in 10 Blockschälchen mit jeweils 1 ml Behandlungsflüssigkeit angesetzt wurden. Hierbei erwies es sich als notwendig, die auf die Blockschälchen aufgelegten Deckscheiben mit etwas Vaseline abzudichten, da anderenfalls die Reproduzierbarkeit der Teste nicht gewährleistet ist. Durchschnittlich zweimal in der Woche wurden die in jedem Schälchen geschlüpften Larven ausgezählt und anschließend zusammen mit der Behandlungsflüssigkeit entfernt, worauf wiederum jeweils 1 ml der gleichen Flüssigkeit frisch aufpipettiert wurde. Die Test- und Kontrollösungen wurden während der ganzen Versuchsdauer von mindestens 3 Wochen bei etwa 5° C aufbewahrt.

Die zur Gewinnung von Wurzeldiffusaten erforderlichen Pflanzen wurden unter Verwendung einer einheitlichen Erdmischung in Blumentöpfen von 12 cm Durchmesser im Gewächshaus kultiviert. Eine Woche vor der WD-Herstellung wurden die Töpfe mit 1-2 L Wasser durchgespült; in den folgenden Tagen wurde durch vorsichtiges Gießen die Wassersättigung des Bodens annähernd aufrechterhalten. Von jeder Topfpflanze stellten wir dann durch langsames Zugießen einer abgemessenen Wassermenge 50 ml Ablaufwasser her. Bei jeder Pflanzenart wurden die Diffusate von mehreren, bei Kartoffeln und Tabak stets von zahlreichen Töpfen miteinander vermischt.

Zur Testung eventuell vorkommender Antischlüpfstoffe stellten wir Misch-

diffusate her, indem das Wurzelablaufwasser der betreffenden Versuchspflanze mit aktivem Wurzeldiffusat der Kartoffelsorte „Aquila" zu gleichen Teilen versetzt wurde. Zur Prüfung gewonnener Hemmstoffpräparate und weiterer Pflanzeninhaltsstoffe bzw. ihrer Derivate wurden diese in Wasser gelöst (bei Säuren und Basen in Form ihrer Salze) und die Lösung im Verhältnis 1 : 1 mit aktivem Aquila-WD gemischt. Für jeden Versuch diente als Kontrolle Aquila-WD, das zu gleichen Teilen mit Aqua dest. versetzt worden war. Zu jeder Testserie verwendeten wir für die Herstellung der Mischdiffusate und der Kontrollösungen Kartoffel-WD gleicher Aktivität. Unter „relativer Hemmwirkung" verstehen wir die Differenz zwischen der relativen Schlüpfrate der Kontrolle (= 100 %) und der relativen Schlüpfwirkung der jeweiligen Testlösung.

ERGEBNISSE

Tabelle II zeigt, daß die Unwirksamkeit der Diffusate verschiedene Ursache haben kann. So ist im Wurzelablaufwasser einiger Pflanzen (z.B. von *Solanum atropurpureum, Nicotiana tabacum, Physalis philadelphica*) ein den Schlüpfvor-

TABELLE II

Schlüpfwirkung von Wurzeldiffusaten verschiedener Solanaceen und von entsprechenden Mischungen dieser Diffusate mit Kartoffel-Wurzeldiffusat.

	Relative Schlüpfwirkung in %	
	WD + H_2O (1 : 1)	WD + Aquila-WD (1 : 1)
1. *Solanum atropurpureum* Schrank	3	4
2. *Datura metel* L.	≈ 1	8-33
3. *Physalis philadelphica* Lam.	< 1	12-20
4. *Solanum aviculare* Forst.	1-67*)	13-73*)
5. *Hyoscyamus falezlez* Coss.	2	20
6. *Cyphomandra betacea* Sendtn.	2-5	21
7. *Withania somnifera* (L.) Dun.	1-11	21
8. *Nicotiana tabacum* „Virgin Gold A"	≈ 1*)	22-40*)
9. *Schizanthus retusus* Hook.	1-2*)	27-105*)
10. *Atropa bella-donna* L.	1-2*)	38-50*)
11. *Hyoscyamus niger* L.	< 1	39
12. *Solanum robustum* H. Wendl.	< 1	82
13. *Solanum melongena* L.	13	87
14. *Solanum abutiloides* Bitter et Lillo	35	96
15. *Lycopersicon peruvianum* Mill.	39	99
16. *Solanum miniatum* Bernh.	2	100
17. *Lycopersicon hirsutum* H. B. et K.	23	119
18. *Cestrum elegans* Schldl.	13	121
19. *Solanum tuberosum* „Aquila"	100	—

*) Pflanzen verschiedenen Alters.

gang hemmendes Agens wirksam. Demgegenüber ist die fehlende bzw. geringe Aktivität der Diffusate von *Solanum miniatum, S. melongena, Cestrum elegans* und weiteren Pflanzen eindeutig auf das Fehlen des „hatching factor" zurückzuführen. Den Schlüpfvorgang hemmende Stoffe treten in den Diffusaten dieser Pflanzen nicht auf. Jedoch war hier, ebenso wie bei mehreren weiteren Mischdiffusaten, eine mehr oder weniger ausgeprägte Schlüpfverzögerung zu beobachten.

Für einige überprüfte Nichtsolanaceen wurden die entsprechenden Befunde in Tabelle III zusammengestellt.

Tabelle III

Wirksamkeit von Wurzeldiffusaten einiger Nichtsolanaceen bzw. von Mischungen dieser Diffusate mit Kartoffel-Wurzeldiffusat

	Relative Schlüpfwirkung in %	
	WD + H_2O (1 : 1)	WD + Aquila-WD (1 : 1)
Asparagus officinalis L.	0	> 100
Avena sativa L.	2-16	—
Cannabis sativa L.	11	> 100
Digitalis purpurea L.	12	79
Glycine soja (L.) Sieb. et Zucc.	3	> 100
Lupinus luteus L.	4	—
Melilotus albus Med.	6	—
Nolana prostrata L.	3-4	100
Tagetes patulus L.	18	58
Trigonella foenum-graecum L.	8	—
Solanum tuberosum „Aquila"	100	—

Nach Triffitt (1930), Ellenby (1945) und Nolte (1958) besitzen die Wurzeldiffusate mehrerer Cruciferen eine schlüpfhemmende Wirkung auf den Kartoffelnematoden, für die der Senfölgehalt (Isothiocyanate) der Diffusate verantwortlich gemacht wird (Smedley, 1939; Ellenby, 1946 und Heeley, 1952). Weiterhin wurden gewisse Schlüpfhemmungen bei verschiedenen *Heterodera*-Arten durch Zugabe von Ferrioxyd (Hurst & Triffitt, 1925; Carroll & McMahon, 1937), von Calciumacetat (O'Brien, Gemmel, Prentice & Wylie, 1939) bzw. von Indolyl-3-essigsäure (Thorne, 1956) zu aktiven Wurzelablaufwässern oder nach Vorbehandlung der Zysten mit Undecylensäure oder Pelargonsäure (Tarjan & Cheo, 1956), mit Harnstoff (Carroll, 1933), mit 1,2-Dibromäthan (Fenwick, 1957) bzw. mit Monobrommethan (Hague, 1959) beschrieben (vgl. Nolte, 1958).

Im folgenden soll über die von uns bisher eingehender untersuchte Schlüpfhemmung durch Wurzeldiffusate von *Nicotiana*-Arten berichtet werden. Im Gegensatz zu anderen Solanaceen wird die schlüpfhemmende Wirkung von Tabak-

Wurzeldiffusaten in ihrer Stärke nur wenig vom Alter und Entwicklungszustand der Pflanzen beeinflußt. Dagegen wurden größere Unterschiede zwischen verschiedenen *Nicotiana*-Arten und ihren Varietäten bzw. Sorten beobachtet (Tab. IV).

Tabelle IV

Relative Schlüpfwirkung der Gemische von Wurzeldiffusaten einiger Nicotiana-*Arten und -Varietäten bzw. Tabaksorten mit Kartoffel-Wurzeldiffusat*

	Relative Schlüpfwirkung in % WD + Aquila-WD (1 : 1)
Nicotiana tabacum L. (Amsterdam)	41
Nicotiana tabacum „Virgin Gold A"	22-40
Nicotiana tabacum „Samsun"	35
Nicotiana tabacum „Havanna IIc"	60
Nicotiana tabacum „Orinoco"	63
Nicotiana tabacum var. gigantea	48
Nicotiana tabacum var. calycina	66
Nicotiana tabacum var. lacerata	79
Nicotiana tabacum var. atropurpureum	100
Nicotiana rustica L. (Dahlem)	100
Nicotiana rustica „Texana"	52
Nicotiana acuminata Hook.	42
Nicotiana glauca Graham	86
Nicotiana quadrivalvis Pursh	61
Solanum tuberosum „Aquila" WD + H_2O (1 : 1)	100

Im allgemeinen wurde die Wirksamkeit der Wurzeldiffusate durch Überprüfung des Ablaufwassers von Topfpflanzen ermittelt. Aber auch das gut gewaschene und in Wasser eingestellte Wurzelsystem von Tabakpflanzen ist über einen längeren Zeitraum in der Lage, Hemmstoffe abzugeben. Zur Klärung der Frage, welche Organe zur Bildung und Ausscheidung der Hemmstoffe bzw. Schlüpfstoffe befähigt sind, wurden neben den Wurzeldiffusaten auch Blattdiffusate sowie ferner die Wurzelablaufwässer von verschiedenen reziproken Pfropfungen zwischen *Nicotiana tabacum* und *Lycopersicon esculentum* überprüft. Diese Untersuchungen führten zu dem Befund, daß Blattdiffusate eine noch stärkere schlüpfhemmende Wirkung besitzen als die Wurzeldiffusate. Allerdings zeigte sich bei der chemischen Aufarbeitung dieser Blattdiffusate (vgl. S. 136), daß die hier vorkommenden Hemmstoffe im wesentlichen anderer Natur sein dürften als diejenigen der Wurzeldiffusate. Die Ergebnisse der Pfropfungsversuche wurden in der Tabelle V zusammengestellt. Hiernach werden erwartungsgemäß von den homoplastischen Pfropfungen Tomate/Tomate Schlüpfstoffe (Tab. V, Nr. 1) und von der Pfropfung Tabak/Tabak Hemmstoffe (Tab. V, Nr. 2 u. 5) ausgeschieden. Die Überprüfung der Pfropfung Tabak/Tomate läßt erkennen, daß bei den Tomatenpflanzen offensichtlich schon die Wurzel allein ohne Beteiligung der

Tabelle V

Wirksamkeit der Wurzeldiffusate reziproker Pfropfungen zwischen Nicotiana tabacum *"Virgin Gold A" und* Lycopersicon esculentum *"Lukullus" sowie relative Schlüpfwirkung einiger Gemische dieser Diffusate*

Tabak-Tomate-Pfropfungen	Geschlüpfte Larven pro Zyste *		
	WD vom: 12.V.59	3.VI.59	3.VII.59
1. WD Tomate/Tomate (+ H_2O, 1 : 1)	14,4 (100 %)	67,4 (100 %)	8,3 (100 %)
2. WD Tabak/Tabak (+ H_2O, 1 : 1)	2,6 (18 %)	4,0 (6 %)	4,7 (57 %)
3. WD Tomate/Tabak (+ H_2O, 1 : 1)	6,8 (47 %)	2,0 (3 %)	1,6 (19 %)
4. WD Tabak/Tomate (+ H_2O, 1 : 1)	20,6 (143 %)	44,8 (66 %)	13,4 (161 %)
5. WD 1 + WD 2 (1 : 1)	4,4 (31 %)	25,5 (38 %)	5,8 (70 %)
6. WD 1 + WD 3 (1 : 1)	14,5 (101 %)	18,0 (27 %)	4,1 (49 %)
7. WD 1 + WD 4 (1 : 1)	10,0 (70 %)	40,8 (61 %)	18,8 (226 %)

* Die in diesem Versuch mit Tomaten-WD erzielten absoluten Schlüpfzahlen sind wesentlich niedriger als bei den Kartoffel-WD-Kontrollen anderer Teste.

oberirdischen Organe zur Bildung der Schlüpfstoffe befähigt ist. Tabak als Reis scheint die normale Schlüpfstoffbildung bzw. -ausscheidung der Tomatenwurzel nicht zu beeinflussen. Hemmstoffe dürften in den Wurzelausscheidungen dieses Pfropfbastardes nicht oder in nur geringen Mengen vorhanden sein (Tab. V, Nr. 4 u. 7). Allerdings ist eine Entscheidung über die Frage, ob die Hemmstoffbildung im Tabakblatt unabhängig von der Tabakwurzel stattfinden kann oder ob in der Tomatenwurzel ein Umbau der vom Tabaksreis eventuell gelieferten Hemmstoffe vor sich geht, auf der Grundlage dieser Versuche noch nicht möglich. Die Wurzeldiffusate der Pfropfung Tomate/Tabak besitzen geringe Schlüpfwirkung, die mit zunehmendem Alter der Pfropfung noch weiter abfällt (Tab. V, Nr. 3). Umgekehrt nimmt der Hemmstoffgehalt der Wurzeldiffusate dieser Pfropfung mit dem Alter des Pfropfbastardes merklich zu (Tab. V, Nr. 6). Diese Pfropfung scheint somit in ihren jüngeren Entwicklungsstadien weder zur Ausscheidung größerer Mengen von Schlüpfstoffen noch von Hemmstoffen befähigt zu sein.

Die mit Tabak-Wurzeldiffusat erzielte Schlüpfhemmung ist reversibel; denn bei einer nach 14tägiger Zwischenwässerung erfolgten Nachbehandlung mit aktivem Kartoffel-Wurzelablaufwasser wurde ein im Vergleich zu den Kontrollen annähernd 100 %iges Schlüpfen der Larven festgestellt. Über die Konzentrationsabhängigkeit der schlüpfhemmenden Wirkung von Tabak-Wurzeldiffusat unterrichtet Tabelle VI. Graphisch dargestellt ergeben diese Werte die für eine Alles-oder-Nichts-Reaktion typische Sigmakurve. Diese und weitere Befunde deuten auf eine kompetitive Hemmung des Schlüpfvorganges durch den im Tabak-Wurzeldiffusat vorhandenen Hemmstoff hin. Dagegen kommt diesem Stoff eine nematizide Wirkung auf geschlüpfte aktive Larven nicht zu. Für solche nicht nematizid wirkenden Substanzen, welche nur den Schlüpfvorgang antagonistisch beeinflussen bzw. die Schlüpfstoffe selbst unmittelbar inaktivieren, wurde als

Tabelle VI

Abnahme der relativen Schlüpfwirkung von Kartoffel-Wurzeldiffusat durch Zusatz ansteigender Mengen von Tabak-Wurzeldiffusat

				Relative Schlüpfwirkung (%)
Kontrolle:	50 H_2O	+ 50	Kartoffel-WD	100
1 Tabak-WD	+ 49 „	+ 50	„	100
5 „	+ 45 „	+ 50	„	77
10 „	+ 40 „	+ 50	„	40-43
20 „	+ 30 „	+ 50	„	34
25 „	+ 25 „	+ 50	„	34
30 „	+ 20 „	+ 50	„	39
40 „	+ 10 „	+ 50	„	24
50 „	+ 0 „	+ 50	„	15-38
75 „	(konz. auf 50)	+ 50	„	20
100 „	(konz. auf 50)	+ 50	„	32
200 „	(konz. auf 50)	+ 50	„	16

Gruppenbegriff die Bezeichnung „Antischlüpfstoffe" vorgeschlagen (Schreiber & Sembdner, 1959).

Die von uns bisher untersuchten Antischlüpfstoffe werden unter den für die Isolierung des „hatching factor" angegebenen Bedingungen (Calam, Raistrick & Todd, 1949) ohne vorherige Ansäuerung der Diffusate von Aktivkohle nicht adsorbiert, so daß auf diese Weise eine Trennung beider Stoffgruppen nachweisbar war. Ein solcher Versuch mit Wurzeldiffusat von *Solanum aviculare,* in dem offensichtlich sowohl Schlüpf- als auch Hemmstoffe vorhanden sind, wird in der Tabelle VII dargestellt.

Tabelle VII

Nachweis von Schlüpfstoffen und Antischlüpfstoffen in Wurzeldiffusaten von Solanum aviculare *Forst.*

		Relative Schlüpfwirkung in %
Kartoffel-WD + H_2O	(1 : 1)	100
Sol. aviculare-WD + H_2O	„	67
Kartoffel-WD + Sol. aviculare-WD	„	108
Kartoffel-WD (+ *K*) *) + H_2O	„	17
Sol. aviculare-WD (+ *K*) + H_2O	„	24
Kartoffel-WD (+ *K*) + Sol. aviculare-WD	„	76
Kartoffel-WD + Sol. aviculare-WD (+ *K*)	„	49
Kartoffel-WD (+ *K*) + Sol. aviculare-WD (+ *K*)	„	13

*) (+ K) = vorher mit Aktivkohle behandelt.

Der im Tabak-Wurzeldiffusat vorkommende Antischlüpfstoff erwies sich als weitgehend thermostabil (5stündiges Kochen), so daß sich das Wurzeldiffusat ohne stärkeren Aktivitätsverlust im Vakuum bis zur Trockene einengen läßt.

Durchgeführte Vorversuche ergaben, daß sich der Antischlüpfstoff aus mit Mineralsäuren angesäuerten Diffusaten (pH ≈ 3) offensichtlich quantitativ, aus neutralem bzw. alkalisiertem Ablaufwasser jedoch nicht mit Diäthyläther extrahieren läßt. Nach Behandlung des Ätherextraktes mit einer wäßrigen Natriumhydrogencarbonatlösung findet sich die Aktivität in der wäßrigen Phase. Die wirksame Substanz wird durch Kochen mit Mineralsäuren (1 Stunde mit 1n HCl, bzw. 5 Stunden mit 0,1n HCl) unter weitgehendem Aktivitätsverlust, durch Kochen mit wäßrigen Alkalien (1 Stunde mit 1n KOH bzw. 5 Stunden mit 0,1n KOH) unter vollständigem Verlust ihrer Wirksamkeit verändert. Weitere unter Mitarbeit von Herrn Dipl.-Chem. G. Osske angestellte Untersuchungen führten zu dem Ergebnis, daß eine Anreicherung des Antischlüpfstoffes durch Behandlung des a n g e s ä u e r t e n Wurzeldiffusates mit Aktivkohle und nachfolgende Elution der Kohle mit Diäthyläther, Aceton-Wasser oder am vorteilhaftesten mit Essigsäureäthylester möglich ist. Das so gewonnene sehr aktive Konzentrat ist in Wasser kaum, in sehr verdünntem wäßrigem Ammoniak jedoch gut löslich. Diese und weitere chemische sowie papier- und säulenchromatographische Untersuchungen deuten darauf hin, daß es sich hier um eine oder mehrere organische Säuren handelt, mit deren Identifizierung bzw. Strukturaufklärung wir zur Zeit beschäftigt sind. Ob den von uns in Diffusaten verschiedener Solanaceen nachgewiesenen Antischlüpfstoffen die gleiche chemische Konstitution zukommt, ließ sich noch nicht klären.

Die naheliegende Vermutung, daß der im Tabak-Wurzeldiffusat vorkommende Antischlüpfstoff mit Nicotin identisch ist, ließ sich somit nicht bestätigen. Darüber hinaus wurde analytisch ermittelt, daß in den Wurzeldiffusaten nur Spuren von Nicotin vorhanden sind, durch welche die Schlüpfhemmung der Ablaufwässer nicht erklärt werden kann. Dennoch zeigten mit reinem Nicotin durchgeführte Testserien, daß auch diesem Alkaloid in höheren Konzentrationen (50 %ige Hemmung bei 100 mg, vollständige Hemmung bei 300 mg Nicotin/L Kartoffel-Wurzeldiffusat) eine Hemmwirkung zukommt (Tab. VIII). Auch hier handelt es sich offensichtlich um eine Alles-oder-Nichts-Reaktion und um eine

Tabelle VIII

Konzentrationsabhängigkeit der reversiblen Schlüpfhemmung durch Nicotin

mg Nicotin L Kartoffel-WD	rel. Schlüpfwirkung bezogen auf Kartoffel-WD in %	Zusätzl. Schlüpfen mit Kartoffel-WD nach anschließ. 14täg. Zwischenwässerung (%)
0	100	11
5	100	
50	100	13
100	46-56	
200	31-33	
300	0-11	
400	0	
500	0	100

reversible Hemmung, denn nach einer 14tägigen Zwischenwässerung der Zysten ließ sich auch nach Vorbehandlung mit höheren Nicotinkonzentrationen ein 100 %iges Schlüpfen der Larven beobachten. Im Gegensatz zu dem natürlichen Antischlüpfstoff aus Tabak-Wurzeldiffusat wirkt das Nicotin nematizid auf geschlüpfte aktive Larven. Inwieweit die starke Hemmwirkung der Blattdiffusate mit ihrem Gehalt an Nicotin bzw. weiteren *Nicotiana*-Alkaloiden zusammenhängt, wird zur Zeit untersucht (vgl. Abb. 1).

Versuche mit weiteren Solanaceen-Alkaloiden zeigten folgende interessante Ergebnisse. Tropin (Tab. IX) und Atropin, das etwa die gleiche molare Konzen-

Tabelle IX

Konzentrationsabhängigkeit der Schlüpfhemmung durch Tropin und ihrer Reversibilität

	Relative Schlüpfwirkung in %	Zusätzl. Schlüpfen mit Kartoffel-WD nach anschließ. 14täg. Zwischenwässerung (%)	
	(I)	(II)	I + II (%)
Kartoffel-WD	100	1-3	101-103
„ + Tropin (0,5 mg/Ltr.)	47		
„ + „ (2,5 mg/Ltr.)	27		
„ + „ (5 mg/Ltr.)	24-41	38	62
„ + „ (25 mg/Ltr.)	31		
„ + „ (50 mg/Ltr.)	30	22	52
„ + „ (100 mg/Ltr.)	15		
„ + „ (250 mg/Ltr.)	5	66	71
„ + „ (500 mg/Ltr.)	9	7	16

trationsabhängigkeit wie Tropin aufweist, führten bereits in niederen Konzentrationen (2,5 mg Tropin/L) zu einer eindeutigen und in höheren Konzentrationen (500 mg Tropin/L) zu einer sogar irreversiblen Schlüpfhemmung. Im Gegensatz hierzu ist Scopolamin, das sich vom Atropin lediglich durch eine zusätzlich vorhandene Epoxygruppe unterscheidet (Abb. 1), in selbst sehr hohen Konzentrationen (2500 mg/L) vollständig unwirksam. Überraschenderweise bewirkt auch Cocain in Konzentrationen bis zu 500 mg/L keine Schlüpfhemmung [1]).

Der vorläufige Befund, daß es sich bei dem Antischlüpfstoff in Tabak-Wurzeldiffusat vermutlich um eine organische Säure handelt, legte nahe, einige natürlich vorkommende Verbindungen dieser Stoffgruppe auf eventuelle Hemmwirkung hin zu überprüfen. Als erstes Ergebnis dieser noch nicht abgeschlossenen Versuche sei mitgeteilt, daß beispielsweise auch den Gibberellinen eine schlüpfhemmende

[1]) Über die mit diesen und weiteren Alkaloiden durchgeführten Untersuchungen wurde an anderer Stelle ausführlicher berichtet (Schreiber & Sembdner, 1960).

Wirkung zukommt [1]). Hervorzuheben ist, daß die in der Literatur bisher mitgeteilten Befunde bezüglich der chemischen Konstitution der Eclepsäure in den wesentlichen Punkten auch auf die Gibberelline zutreffen. Für die Gibberellinsäure (Abb. 2) und für die Eclepsäure wurden die entsprechenden Daten in der Tabelle X vergleichend zusammengestellt. Von der Gibberellinsäure ($C_{19}H_{22}O_6$)

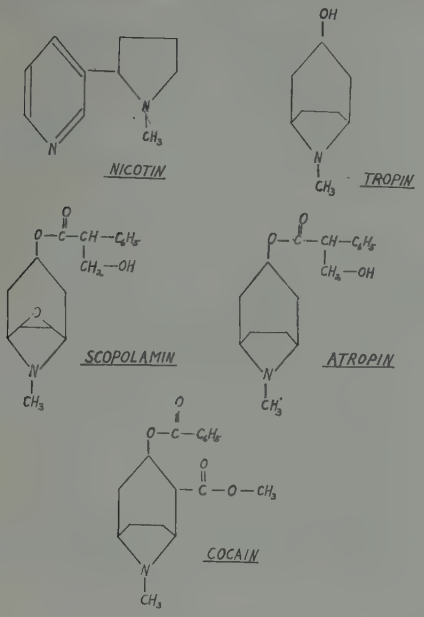

Abb. 1. Formelbilder von Nicotin, Tropin, Scopolamin, Atropin und Cocain.

ausgehend läßt sich die für die Eclepsäure ermittelte Zusammensetzung $C_{19}H_{20}O_8$ durch Epoxydierung einer Doppelbindung sowie durch Annahme eines weiteren Äthersauerstoffes, eventuell in Form einer zweiten Epoxygruppe, formell ablei-

Tabelle X

Vergleich konstitutiver Ähnlichkeiten zwischen Gibberellinsäure und Eclepsäure

	Gibberellinsäure	Eclepsäure
Summenformel	$C_{19}H_{22}O_6$	$C_{19}H_{20}O_8$
Molekulargewicht	errechnet 346	bestimmt 300-400
γ-Lactongruppen	1	1
Carboxylgruppen	1	1
freie Hydroxylgruppen	2	1-2
Doppelbindungen	2	1

[1]) Umfangreichere Untersuchungen über den im Vergleich zu anderen Schlüpfversuchen abweichenden Verlauf der Gibberellinteste werden demnächst veröffentlicht.

ten. An dieser Stelle sei nochmals auf die unterschiedliche Wirksamkeit von Atropin und dem eine Epoxygruppe enthaltenden Scopolamin (Abb. 1) sowie auf die bekannte Aktivierung der natürlichen Schlüpfstoffe durch Peroxyde (ELLENBY, 1958) hingewiesen. Denkbar ist, daß Peroxyde eine Epoxydierung inaktiver Vorstufen der Schlüpfstoffe oder sogar eine Epoxydierung von Hemmstoffen be-

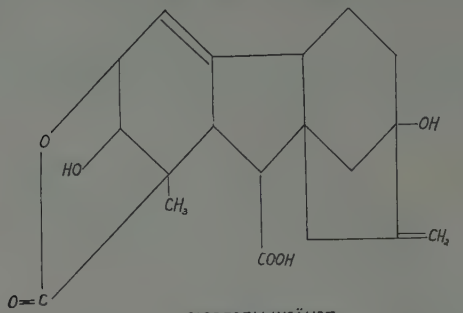

Abb. 2. Formelbild der Gibberellinsäure ($C_{19}H_{22}O_6$)

wirken, die mit einer Wirkungsumkehr verbunden sein könnte. Auf derartige konstitutive Beziehungen zwischen Schlüpfstoffen und Antischlüpfstoffen deutet auch die offensichtlich kompetitive Wechselwirkung zwischen Kartoffel- und Tabak-Wurzeldiffusaten hin. Die weitere experimentelle Überprüfung dieser Arbeitshypothese wurde nach verschiedenen Richtungen hin in Angriff genommen.

SUMMARY

The hatching and antihatching effects of the root diffusates of various plants on the eggs of the potato root eelworm, Heterodera rostochiensis *Woll.*

The hatching activity of root diffusates of various Solanaceae and a few other plants towards eggs of the potato root eelworm, *Heterodera rostochiensis* Woll. was tested. The diffusate of many plants, even of a few host plants, had no activity. Sometimes this was due to the absence of a hatching factor and sometimes to the presence of inhibitors. This suggests that hatching factor and inhibitors may often occur together. So far detailed tests have been done only with inhibition due to diffusates of *Nicotiana spp.* From chemical studies made, the inhibitors in these root diffusates appear to be one or more organic acids, which have a completely reversible effect and exert no nematicidal action on active, hatched larvae. Furthermore, the concentration at which inhibition occurred was determined. The effects of the Solanaceous alkaloids Nicotine, Tropine, Atropine and Scopolamine were also tested. Similarities in chemical structure pointed to a relationship between the inhibition of Gibberellins and the activity of Eclepic acid.

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DER EINFLUß EINIGER GEWÄCHSE AUF DIE POPULATION VON *MELOIDOGYNE HAPLA*

VON

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Das Vorkommen des Wurzelgallenälchens, *Meloidogyne hapla* Chitwood, 1949, ist aus Gärtnereien, von Kleinlandflächen und anderen Grundstücken in West-Europa bekannt. Dieses polyphage Älchen kommt auf leichten Sandböden im Freiland vor und kann offenbar bei Gemüse- und Gewürzpflanzen, Kartoffeln, Rüben u.a. eine Wachtumsverzögerung verursachen (autores diversi).

Über den Einfluß von Fruchtwechsel auf die Populationsdichte dieses Nematoden ist wenig bekannt. Nach OOSTENBRINK (1953) wird die Population besonders von der Kartoffel vermehrt, aber von Mais gedrückt. BROWN (1957) weist darauf hin, daß besonders nach Leguminosen und Gemüsearten Befall auftritt. HAHN (1958) meldet einen starken Befall nach Mais, der jedoch der Verunkrautung mit *Galinsoga* zugeschrieben wird.

Weitere experimentelle Daten wurden in der Literatur nicht gefunden.

UNTERSUCHUNGEN

Eine genauere Kenntnis über den Einfluß verschiedener Gewächse auf die Population wurde experimentel in zwei Feldversuchen gewonnen, nämlich auf einem leichten Tonboden („E") und auf einem als Gemüsegarten genutzten leichten, sauren Sandboden („W"). Beide Versuche waren in den letzten Jahren mit für *M. hapla* anfälligen Pflanzen bebaut worden. Durch eine Bodenuntersuchung war festgestellt worden, daß der genannte Nematode auf beiden Feldern verbreitet war.

Auf dem Versuchsfeld „E" wurden im Jahre 1955 auf zwei Parzellen, A und B, in vergleichbarer Weise mit vier Wiederholungen Erbse (*Pisum sativum* L.), Rübe (*Beta vulgaris* L.), Gras/Klee, Kartoffel (*Solanum tuberosum* L.), Möhre (*Daucus carota* L.), Zichorienwurzel (*Cichorum intybus* L.), Hafer (*Avena sativa* L.), Roggen (*Secale cereale* L.) und Gerste (*Hordeum vulgare* L.) angebaut. Auf jeder Teilfläche wurde die Anzahl der *Meloidogyne* Larven je 100 ml Boden festgestellt (s'JACOB & STEMERDING, 1956).

Fig. 1 demonstriert die Gesamtresultate der vier Wiederholungen. Die Zahl der Larven im Boden war hoch nach Erbsen, Rüben, Gras/Klee und Kartoffeln, dann folgten Möhren und Zichorienwurzeln, während die Larvenzahl nach Getreide am niedrigsten lag.

Der Einfluß der Gewächse auf die Population von *M. hapla* ist durch eine Bodenuntersuchung nicht immer festzustellen, da es sich hier um Endoparasiten handelt, die mit den Wurzeln großenteils entfernt werden oder die sich nach der Ernte noch längere Zeit innerhalb der Wurzelreste aufhalten können. Die Möglichkeit besteht, daß dieser Faktor besonders bei den Möhren und Zichorienwurzeln eine Rolle gespielt hat, da gerade bei diesen Pflanzen große Mengen noch frischer Wurzeln geerntet wurden.

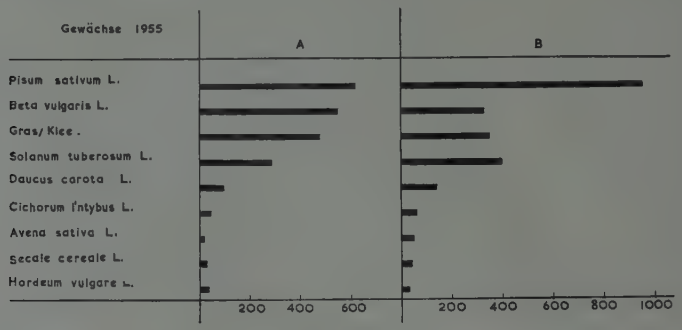

Fig. 1. Versuchsfeld 'E'. Zahl der *Meloidogyne*-Larven pro 400 ml. Erde nach Gewächsen 1955. Zwei gleich bebaute Parzellen A und B, auf denen jedes Gewächs mit vier Wiederholungen angebaut wurde.

Auf dem Felde „W" wurde im Jahre 1958 ein Versuch angelegt, bei dem drei verschiedene Bodenbehandlungen angewandt wurden, die jedoch die Untersuchungen der Wirtspflanzen nicht definitiv beeinflußt haben und die darum weiter nicht besprochen werden. Die folgenden Pflanzen wurden auf den verschieden behandelten Parzellen A, B und C in vergleichbarer Weise mit zwei Wiederholungen angebaut: Kartoffel (*Solanum tuberosum* L.), Rübe (*Beta vulgaris* L.), Erbse (*Pisum sativum* L.), Gartenbohne (*Phaseolus vulgaris* L.), Möhre (*Daucus carota* L.),

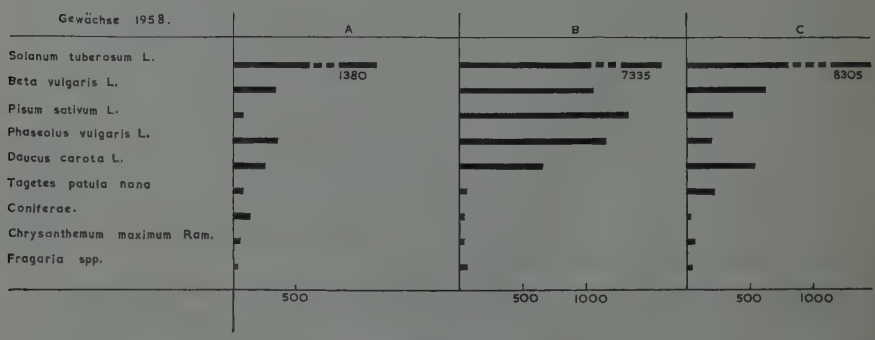

Fig. 2. Versuchsfeld 'W'. Zahl der *Meloidogyne*-Larven pro 200 ml Erde. Drei gleich bebaute Parzellen A, B und C, auf denen jedes Gewächs mit zwei Wiederholungen angebaut wurde.

Studentenblumen (*Tagetes patula nana*), einige Coniferen-Arten, Chrysanthemen (*Chrysanthemum maximum* Ram.) und Erdbeere (*Fragaria* spp.).

Fig. 2 demonstriert die Zahl der *Meloidogyne*-Larven je 200 ml Erde, d.h. die Gesamtresultate von zwei Wiederholungen. Der Einfluß der verschiedenen Gewächse war auch hier groß. Besonders nach Kartoffel, Rübe, Erbse, Gartenbohne und Möhre wurde eine hohe Population erreicht. Auf der unbehandelten Parzelle A, wo die Gewächse sehr schwach entwickelt waren, sind die Nematodenpopulationen am wenigsten gestiegen. Die Vermehrung ist also teilweise abhängig gewesen von diesen noch hinzukommenden Faktoren.

SCHLUSSFOLGERUNG

Die vorgenannten Versuche weisen darauf hin, daß vor allem Kartoffeln, aber auch Rübe, Möhre und verschiedene Leguminosen zu den wirksamen Wirtspflanzen von *M. hapla* gerechnet werden müßen und eine hohe Population aufbauen. Getreide und einige andere Pflanzen haben offenbar die Population gedrückt.

SUMMARY

The influence of certain plants on the population of Meloidogyne hapla

The results of two field experiments show a strong influence of certain crops on the population of *Meloidogyne hapla*. Potatoes, beets, carrots and a number of leguminous plants appear to be efficient hosts, which build up a high population of the nematode. Cereals and some other crops evidently suppressed the population.

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ÉTUDE HISTOLOGIQUE DE NÉMATOCÉCIDIES EN MICROSCOPIE DE FLUORESCENCE

PAR

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Les techniques histologiques habituellement utilisées en botanique ne donnent pas toujours satisfaction lorsqu'on les applique à l'étude des nématocécidies. Ceci est dû à plusieurs raisons: lenteur des diverses opérations lorsqu'il s'agit de préparer le matériel, si l'on utilise le microtome à paraffine; difficulté de maintenir *in situ* dans les coupes minces les Nématodes, qu'ils soient à l'état d'oeuf, de larve ou d'adulte; enfin nécessité dans laquelle on se trouve de ne pas vider les coupes, ce qui rend celles-ci peu lisibles quand elles sont faites à main levée.

La propriété que possèdent les tissus lignifiés d'être naturellement fluorescents sous l'excitation d'une source de rayons ultraviolets et leur aptitude à adsorber de façon élective certains fluorochromes, nous ont incité à rechercher une méthode d'exécution plus rapide pour examiner les modifications résultant de la présence de Nématodes cécidogènes (LANGERON, 1942; HOMEYER, 1953).

Les études ont porté sur des tumeurs radiculaires induites chez différents végétaux par *Meloidogyne incognita* (Kofoid et White).

DESCRIPTION DE LA TECHNIQUE

Les coupes histologiques sont exécutées sur du matériel frais ou conservé dans une solution de formol à 4 %, soit à main levée, soit à l'aide d'un microtome à congélation. Cette dernière méthode prévaut par sa rapidité et par le fait qu'elle permet d'exécuter des coupes d'épaisseur uniforme (40 μ et moins), d'orientation constante aussi bien dans le plan longitudinal que transversal.

Le matériel utilisé a été de préférence la tomate, *Lycopersicum esculentum,* où l'on a examiné à la fois les structures saines et des galles de plus ou moins grande importance. Des épreuves de contrôle ont parallèlement été exécutées sur *Beta vulgaris, Anthemis maritima, Brassica oleracea* et *Cucurbita pepo.*

L'observation des structures, colorées ou non, est faite à l'aide d'un microscope ordinaire et en lumière épiscopique. Un dispositif „ZEISS" comprenant: la microlampe à vapeur de mercure HBO-74 et le verre bleu BG-12 monté dans le premier porte filtre destiné à sélectionner la lumière ultraviolette, est utilisé comme source d'excitation de la fluorescence primaire ou secondaire des préparations. Des filtres d'arrêt OG-5 sont placés sur les oculaires.

Fig. 1. Racine de tomate, structures normales. (× 22).

Fig. 2. Ponte de *Meloidogyne* "in situ" à plus fort grossissement. (× 50).

J. R. LE BERRE & M. RITTER: *Étude histologique de Nématocécidies.*

Fig. 3. Racine de tomate a attaquée. Coupe longitudinale dans une demi galle (le cylindre central occupe l'extrême gauche de la photo). (× 8).

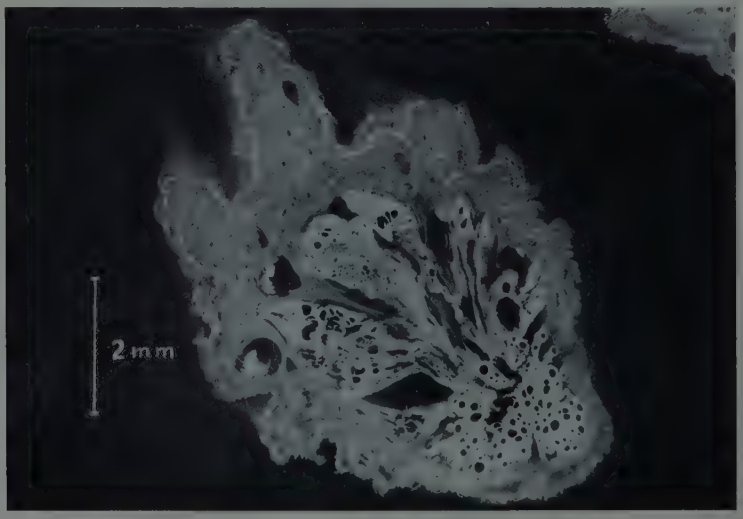

Fig. 4. Racine de tomate attaquée: Structure dissymétrique des expansions lignifiées dans une galle. (× 12).

La fluorescence secondaire est obtenue en traitant les objets, comme pour une coloration histologique, par des solutions très diluées de divers fluorochromes. Les meilleurs résultats pour chacun d'eux ont été obtenus avec les concentrations suivantes:

Série des Acridines	:	Phosphine 3R au 1/5000
	:	Coriphosphine O à 1/10000
	:	Orange d'Acridine 1/10000
Série des Thiazols	:	Thioflavine S au 1/10000
Série des Quinoleines	:	Sulfate de Berbérine au 1/5000
Série des Phénylméthylanes	:	Auramine 1/10000
	:	Rhodamine au 1/20000

Il n'est pas nécessaire de fixer les coupes, ni de les vider par immersion dans l'hypochlorite de soude. Un simple trempage d'une durée de 10 à 20 minutes dans chaque solution suffit. Puis le matériel est rincé à l'eau distillée jusqu'à épuisement de toute diffusion et les coupes montées à la glycérine peuvent être examinées immédiatement.

La fluorescence secondaire peut se maintenir plus d'une année lorsque les préparations sont lutées et gardées à l'obscurité.

En même temps que celles traitées aux fluorochromes, un certain nombre de coupes de reférence ont toujours été colorées selon des méthodes de type plus classique. Les meilleurs résultats ont été obtenus avec les doubles colorations: carmin aluné-vert d'iode, bleu de méthylène aluné-rouge de ruthénium.

En règle générale, la microscopie de fluorescence nécessite l'emploi de filtres d'arrêt. Ceux-ci, qui protègent l'oeil d'un excès de radiations directes, violettes et ultraviolettes, permettent en même temps d'obtenir des images plus fines et plus brillantes. Toutefois l'absence de filtres ne paraît pas gêner l'observation, les radiations utilisées n'allant pas très loin dans l'ultraviolet; dans ce cas, on obtient une plus grande diversité de teintes dans la fluorescence secondaire en fonction du fluorochrome employé. Ainsi la Rhodamine qui donne au niveau du xylème et sans filtre d'arrêt une intense fluorescence secondaire dans le rouge, voit cette fluorescence virer au jaune orangé par interposition des filtres OG-5.

RÉACTION DES DIFFÉRENTS TISSUS DE LA RACINE SAINE OU ATTAQUÉE PAR MELOIDOGYNE INCOGNITA

Après une étude systématique de l'action des 7 fluorochromes précédemment cités sur les divers tissus de la racine, ainsi d'ailleurs que sur les différents stades évolutifs des Nématodes présents dans les galles, les quelques faits précis suivants peuvent être dégagés (Fig. 1):

1. Chez la Tomate: La fluorescence secondaire du xylème, de l'épiderme, et probablement de tout tissu lignifié ou subérifié est particulièrement vive et parfaitement distincte selon la nature du tissu et le fluorochrome utilisé.

2. Le phloeme n'a pas de fluorescence primaire, il réagit faiblement avec la Coriphosphine et pratiquement pas avec les autres fluorochromes essayés.

3. Le parenchyme cortical ainsi que les travées parenchymateuses du cylindre central présentent une fluorescence assez bien discernable avec la Coriphosphine, l'Auramine et la Thioflavine, caractérisée par des limites cellulaires brillantes qui permettent de suivre, partout où il y en a, ce type de structure.

4. L'assise endodermique est particulièrement visible par l'emploi de la Coriphosphine et de l'Auramine. On verra dans les lignes suivantes que la mise en évidence de cette assise est importante dans l'étude du processus de la prolifération cellulaire dans la cécidogénèse.

5. En règle générale, l'action caractéristique des différents fluorochromes sur chaque tissu de la racine, se retrouve chez des plantes appartenant à des familles diverses. Toutefois, l'intensité et la persistance de la fluorescence montrent des différences: elles dépendent à la fois de l'espèce végétale et du fluorochrome utilisé. Ainsi, les structures lignifiées du chou: *Brassica oleracea,* ont une fluorescence médiocre avec la Thioflavine ou la Coriphosphine et il est nécessaire de la renforcer, en faisant agir un second fluorochrome, par exemple: la Rhodamine.

6. Les individus de *Meloidogyne* coupés *in situ* en même temps que les galles qui les entourent, présentent parfois des fluorescences particulières permettant une excellente mise en évidence des tronçons de larves ou de femelles ainsi que les oeufs (Fig. 2).

La fluorescence des oeufs par exemple, est très marquée pour la Coriphosphine (jaune vif), la Phosphine (jaune terne), et la Rhodamine (jaune orange), elle est moins accusée avec l'orange d'Acridine et la Thioflavine et presque nulle pour l'Auramine et le Sulfate de Berbérine.

RÉSULTATS D'OBSERVATION ET DISCUSSION SUR LA CÉCIDOGÉNÈSE

L'étude histologique des galles provoquées sur diverses plantes par des Nématodes appartenant au genre *Meloidogyne,* a fait l'objet d'un certain nombre de travaux remontant généralement à une époque assez ancienne où l'on ne soupçonnait pas la diversité réelle des espèces réunies sous le nom d'*Heterodera radicicola* ou d'*Heterodera marioni.* Ceci peut expliquer les divergences d'observations et d'interprétations rencontrées dans la littérature.

La présence de ces Nématodes dans la racine y provoque deux réactions principales: la déformation des éléments conducteurs accompagnée d'une prolifération de tissus avoisinants et l'apparition de cellules géantes polynuclées très caractéristiques. La plupart des travaux classiques insistent sur ce dernier phénomène. Nous nous attacherons plutôt ici au premier, notre technique d'observation par microfluoroscopie n'étant pas vraiment cytologique et s'appliquant plus à l'étude histologique des nodosités.

Dans le cas de *Meloidogyne incognita,* espèce très fréquente, très polyphage et prédominante dans les régions méditerranéennes, les galles deviennent énormes chez beaucoup d'hôtes et présentent des caractères histologiques communs, quelle

que soit la famille botanique. Elles montrent un développement dissymétrique lié à la localisation des Nématodes dans la zone périphérique. Dans le secteur opposé au point de pénétration des larves, la croissance et l'ensemble des structures végétales restent normaux (Figs. 3 et 4).

Au contraire, en face de la cavité ou des cellules géantes provoquées par la présence de *Meloidogyne incognita*, une activité différenciatrice des formations libéro-ligneuses paraît exacerbée, des travées lignifiées et des masses de liber se dirigent vers les Nématodes en s'épanouissant plus ou moins en champignon. Ces proliférations paraissent converger vers chaque Nématode à partir de la zone la plus proche de l'assise libéro-ligneuse. Ce phénomène qui est moins orienté et plus anarchique dans les galles âgées renfermant de nombreux individus, se manifeste aussi bien dans le plan longitudinal que dans le plan transversal.

Si, en coupe transversale, certains amas de cellules ressemblent à des faisceaux vasculaires parallèles au cylindre central, par contre, les coupes longitudinales précisent qu'il n'existe pas nécessairement de continuité entre ces éléments vasculaires souvent courts et réticulés, et les vaisseaux parfaits. Ces structures ne sont pas sans rappeler celles que l'on rencontre dans des cultures de tissus végétaux faites en présence de faibles doses d'acide indole-acétique (GAUTHERET, 1955).

Parallèlement, le parenchyme interstitiel prend un développement considérable et le parenchyme cortical s'épaissit en se désorganisant, les cellules perdent leur disposition en lamelles concentriques tandis que l'endoderme peut disparaître dans le cas de galles énormes. Sous l'épiderme le liège s'épaissit et l'on voit apparaître des plages liègeuses autour des cavités pleines d'oeufs situées dans le parenchyme.

Ces phénomènes s'observent particulièrement bien chez la tomate lorsque les coupes sont traitées à la Coriphosphine.

Nos observations concordent dans leurs grandes lignes avec celles des auteurs qui ont étudié ces phénomènes, en particulier: TREUB (1887), BEILLE (1898), MOLLIARD (1900), KOSTOFF & KENDALL (1931), CHRISTIE (1936), etc. Le travail d'ensemble le plus récent, celui de GARRIGUES (1951) analyse tous les travaux antérieurs et décrit quelques cas particuliers où les modifications que subit la structure générale semblent moins accusées tandis que le développement des cellules géantes est plus considérable. Selon cet auteur, les expansions ligneuses sembleraient organisées, se comportant en vaisseaux nourriciers des zones de prolifération et des cellules géantes. Cette réaction sensiblement différente de celle que nous observons peut être le fait d'une autre espèce de *Meloidogyne,* en effet *M. incognita* ne se rencontre pas en plein air dans la région parisienne. Les processus d'induction sont probablement susceptibles de différer selon les espèces de nématodes considérées, comme en témoigne l'expression quelque peu variable des réactions morphologiques de certains hôtes communs à plusieurs d'entre elles.

Dans ce travail nous avons appliqué une méthode d'étude nouvelle à des galles déjà agées, renfermant parfois un assez grand nombre de parasites. Pour mieux préciser les phénomènes il sera nécessaire de suivre la formation de la galle dès ses premiers stades et chez des radicelles selon la technique de Christie.

ZUSAMMENFASSUNG

Histologische Studien über Nematodengallen mit Hilfe der Fluorescenz-Mikroskopie.

Eine schnelle histologische Methode zur Demonstration der verschiedenen Wurzelgewebe und der in ihnen enthaltenen Nematoden wird beschrieben.

Die Gallen werden mit dem Gefriermikrotom geschnitten und während einiger Minuten in sehr verdünnten Lösungen von fluorochromen Substanzen gefärbt. Die verholzten oder verkorkten Gewebe zeigen dann eine sehr ausgeprägte Färbung, wenn sie U.V.-Strahlen ausgesetzt und bei auffallendem Licht untersucht werden. Auch die Nematoden sind sehr leicht in situ zu sehen. „Coriphosphine" gibt die besten Resultate.

Beim Studium von Gallen, die an Tomaten und anderen Pflanzen durch *Meloidogyne incognita* verursacht worden sind, zeigte diese Methode den regellosen Charakter der zellulären Auswüchse, besonders derjenigen des Xylems. Diese Auswüchse scheinen ihren Ursprung in der Schicht der libriformen Holzfasern zu haben. Sie konvergieren ebenso oft in der transversalen wie in der radiären Ebene in Richtung gegen die im Parenchym befindlichen Nematoden.

Die sehr variierenden Beobachtungen der verschiedenen Verfasser, die diese Erscheinungen studiert haben, zeigen, dass die Reaktionen der Pflanze sich hauptsächlich nach der *Meloidogyne*-Art, die sie hervorruft, richten. Die allgemeinen Kennzeichen dieser Reaktionen scheinen somit mehr von der Nematoden-Art als von der Wirtspflanze abhängig zu sein.

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